Contract No. DA-18-108-AMC-242(A)

FINAL REPORT

Nonlabelled Compounds for Research Application

Covering the Period
27 June 1963 to 1 July 1964

MELPAR INC
A SUBSIDIARY OF WESTINGHOUSE AIR BRAKE COMPANY
3000 ARLINGTON BOULEVARD FALLS CHURCH, VIRGINIA

Melpar, Inc.
3000 Arlington Boulevard
Falls Church, Virginia

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NONLABELLED COMPOUNDS FOR RESEARCH APPLICATION

Prepared by

J. M. Augl

L. M. Kndley

H. E. Podall

August 1964

ABSTRACT

Fifty-seven compounds were prepared and characterized under this contract of which twenty-eight were delivered to USACRDL. None of the compounds delivered were commercially available. In some instances the method of preparation and the physical constants are reported in the literature, while in other cases analogous procedures were applied to the synthesis of the desired compounds. A number of new compounds were prepared which required research effort to develop a satisfactory synthesis route.

The synthesis of a number of new compounds was started, but further research and development effort is required to obtain final results. The research on these compounds to date is discussed along with proposed routes for the synthesis of model compounds.

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd)

1. INTRODUCTION AND STATEMENT OF TASK

This is the Final Report of the work accomplished on Contract No. DA 18-108-AMC-242(A). The Contracting Officer at CRDL was Dr. Robert I. Ellin; the Melpar Principal Investigator was Dr. H. E. Podall.

The task of this project was to prepare various organic and/or inorganic compounds that were not commercially available, as called for by the Contracting Officer. The quantities prepared were not to exceed 1 kilogram in weight. Quantitative analyses and other analytical data were supplied with each compound as evidence of its composition, structure, and purity. Table I lists the compounds prepared and delivered during the contract period. Compounds, whose syntheses have been started but which require further research and development effort, are listed in table II. The detailed experimental procedures for the synthesis of each compound prepared under this contract and delivered to CRDL are presented in an appendix.

TABLE I

COMPOUNDS PREPARED AND DELIVERED

Compound	Melpar Code	Amount Delivered
methyl phenylcyclobutylglycolate	110	101 g
3-hydroxy-5,8,10-trisulfonic acid of pyrene, Na-salt	120	5 g
pyrene sulfonyl chloride	121	0.4 g
chloroacetylglycylglycine methyl ester	130	2 g
chloroacetylglycylglycine ethyl ester	131	2 g
chloroacetylglycylglycine n-propyl ester	132	2 g
1-(2-oxypyrrolidino)-4-pyrrolidino-butyne-2	150	2 g
1-(2-oxypyrrolidino)-4-pyrrolidino-butyne-2	150	33 g[a]

TABLE I (Continued)

Compound	Melpar Code	Amount Delivered
1,3bis(triethylammonium) propane dibromide	171	5 g
4-trimethylammonium methyl crotonate bromide	172	9 g
4-dimethylsulfonium methyl crotonate bromide	173	3.5 g
4-trimethylammonium methyl butyrate bromide	174	20 g
4-dimethylsulfonium methyl butyrate iodide	175	10 g
"TPCK"	180	4.5 g
α-chlorobenzaldoxime	210	9.6 g
α-chloro-2-pyridine aldoxime hydrochloride	220	16 g
α-chloro-2-pyridine aldoxime methyl chloride	221	3.7 g
2-fluorobenzaldoxime	222	12 g
α-chloro-α-nitrosoacetone	230	3.7 g
mono-isonitrosoacetone	231	35 g
pyridinium acetophenoneoxime chloride	250	2.5 g
3-bromobutyryl bromide	280	12 g
4-bromobutyryl bromide	281	35 g
4-chlorobutyryl chloride	282	100 g
3-chlorobutyryl chloride	283	10 g
5-bromovaleryl bromide	284	10 g
3-bromopropionyl bromide	285	9 g
N-benzoyl-l-tyrosine-p-nitroanilide	290	0.5 g
glycylphenylalanine-p-nitroanilide	291	50 mg
glycylphenylalanine-p-nitroanilide	291	1 g

TABLE I (Continued)

Compound	Melpar Code	Amount Delivered
p-aminobenzyl cellulose	320	b
p-aminobenzyl cellulose	320	49 g[c]

Legend:

a. An additional request by the USCRDL

b. Three development samples of p-aminobenzyl cellulose prepared by changing the procedure in each run.

c. USACRDL's decision was to scale up sample B which was the most satisfactory run of b.

TABLE II

COMPOUNDS WHICH REQUIRE FURTHER R & D EFFORT

Compound	Melpar Code	Status
5-amino-2-picoline	141	5 g[a]
5-α-chloroacetoxy-pyridine	147	b
6-dimethylamino-pyridine-2-aldoxime	149	c
α-bromobenzaldoxime	211	d
6-N,N-dimethylamino-7-methylcarbamyl-phenanthridine	263	e
N-[7-(dimethylamino)-phenanthridyl]-N'(dimethyl)-urea	264	f
2-amino-3N,N-dimethylaminofluorenone-9	300	g
MEL 340	340	h
p-methyl-N-(p-cyclohexylbenzyl)-tropylbenzoate halide	341	i

Legend:

a. Prepared 5 g for conversion at a later date to another compound desired by the USACRDL.

b. Synthesis Research. Two precursors have been synthesized thus far:

(1) α-picoline-5-sulfonic acid.
(2) 5-hydroxy-α-picoline.

c. Desired compound could not be isolated.

d. Several experiments to synthesize this compound were unsuccessful, however, a new compound having two bromine atoms in the side chain has been isolated (see text).

e. Several precursors have been synthesized (see text).

f. Synthesis research. Five precursors have been synthesized and well characterized:

(1) 2-nitro-3-bromo-fluorenone.
(2) 2-nitro-3-dimethylamino-fluorenone.
(3) 2-amino-3-dimethylamino-fluorenone.
(4) 2-nitro-3-dimethylamino-9-hydroxy-fluorene.
(5) 6-dimethylamino-7-nitro-phenanthridine.

g. For conversion to Mel-263 and Mel-264.

h. Several precursors have been synthesized (see text).

i. Two precursors have been synthesized:

(1) p-methylbenzoyl chloride.
(2) tropyl-p-toluate hydrochloride.

2. GENERAL SYNTHESIS EFFORT

The compounds as specified by the Contracting Officer to be synthesized under this contract were divided into two general categories as follows:

a. Compounds not commercially available but described in the literature. In some instances the specific compound with its physical constants and a detailed method of preparation was reported in the literature. In other cases very similar homologs or analogs of the desired compound were described, and the methods used for their preparation were entirely applicable to the synthesis of the desired compound.

b. New compounds requiring research effort to develop a satisfactory synthesis route. Included in this category were the following:

(1) Investigation of the α-picoline-butyl nitrite route for the preparation of pyridine-2-aldoxime and extension of this method to the preparation of various pyridine-2-aldoxime derivatives.

(2) Attempts to improve the yield of phenylcyclobutylglycolic acid in the oxidation step.

(3) Study of the feasibility of electrolyte oxidation of 1-cyclobutyl-1-phenyl-2-propyne-1-ol to the glycolic acid.

(4) Attempts to prepare α-bromobenzaldoxime and elucidation of the nature of the product resulting from the attempted α bromination of benzaldoxime.

(5) Preparation of 2-(α-chloro)pyridiniumaldoxime methchloride.

(6) Preparation of new quaternary ammonium and ternary aliphatic sulfonium compounds.

(7) Attempts to prepare certain tropine derivatives.

(8) Development of an improved process for preparing p-aminobenzyl cellulose for use an an ion exchange material.

(9) Synthesis of certain specific fluorene and phenanthridine derivatives.

(10) Development of an improved procedure for the synthesis of glycylphenylalanine -p- nitroanilide.

(11) Design of a possible route for the synthesis of 5-α-chloro-acetoxypyridine aldoxime methiodide. This included proposed model compound synthesis and development of improved methods for preparation of key starting materials.

3. MEETING WITH CONTRACTING OFFICER AND CONSULTANT

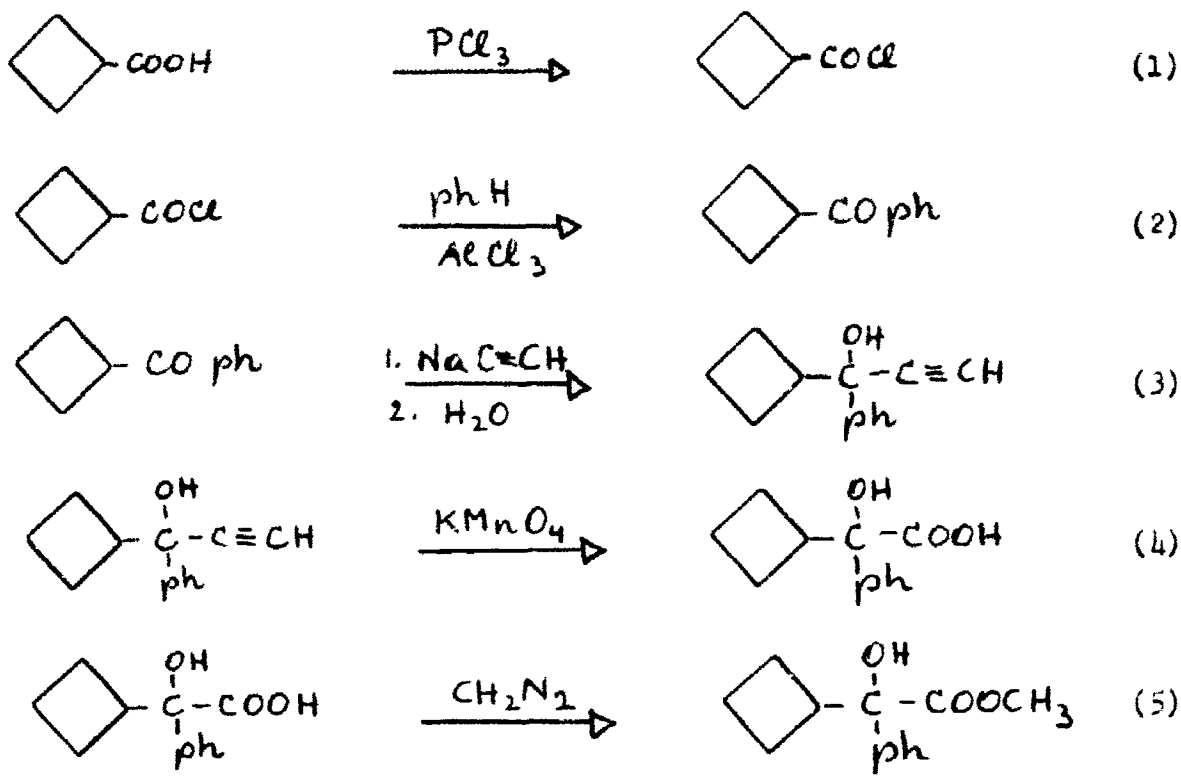
On November 12, 1963, a meeting was held with Dr. R. I. Ellin of the USACRDL, Edgewood, Maryland, and Dr. R. Levine, Professor of Organic Chemistry, University of Pittsburgh, at Melpar, Inc., Falls Church, Virginia, to review plans for this project. Major emphasis was given to possible methods for preparing substituted pyridine-2-aldoximes. It was generally agreed that the use of model compounds would serve to most quickly delineate the feasibility of various methods for preparing the desired compounds.

4. DISCUSSION AND RESULTS*

4.1 Methyl Cylobutylphenyl Glycolate (MEL-110)[1,2]

Overall yields of the multistep synthetic route have not been high, but the poor yield of final product has been due to the inefficiency of oxidation of 1-cylobutyl-1-phenyl-2 propyne-1-ol (step (4) in the synthesis below). Though some research has been carried out in an attempt to improve the yield of acid from permanganate oxidation, it is apparent that further exploration of alternate oxidation procedures would be worthwhile, particularly if the preparation of large quantities of this material is to be economically attractive.

Reactions:

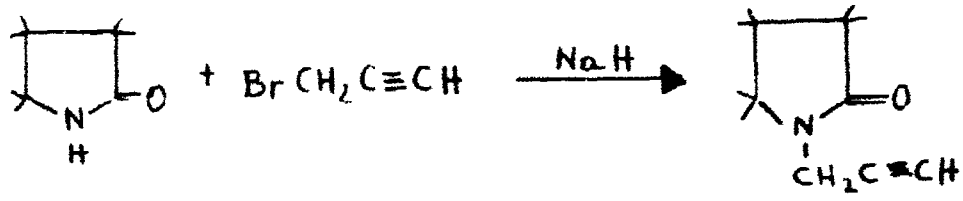


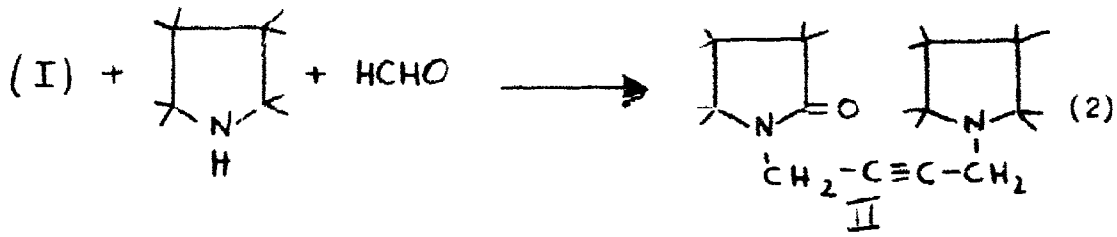
*The results will be discussed in chronological order of delivery.

Cyclobutanecarbonyl chloride was prepared from the corresponding acid and PCl_3 without solvent. The product was isolated by fractional distillation. This compound was reacted with benzene in a Friedel-Crafts-reaction using $AlCl_3$ as the catalyst. The resulting cyclobutyl phenyl ketone was obtained by vacuum distillation. This compound was reacted with sodium acetylide in liquid ammonia and subsequently hydrolyzed with water to give cyclobutyl-1-phenyl-2-propyne-1-ol, which was isolated by vacuum distillation. Cyclobutylphenyl-glycolic acid was obtained from this propynol by oxidation with $KMnO_4$ in aqueous solution, which on treatment with diazomethane gave the desired methylester.

4.2 1-(2-Oxypyrrolidino)-4-Pyrrolidino-Butyne-2 (Mel-150)[3]

Reactions:





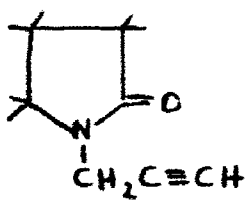
The sodium salt of 2-pyrrolidinone (prepared from pyrrolidinone and NaH) was reacted with propargylbromide to give 1-propargyl-2-pyrrolidinone (I). Pyrrolidine, paraformaldehyde, and (I) were refluxed in dioxane. The resulting 1-(2-oxypyrrolidino)-2-pyrrolidino-butyne-2 (II) was purified by high-vacuum distillation.

In a second preparation, the yield was improved using column chromatography for the purification of (II) instead of ordinary vacuum distillation.

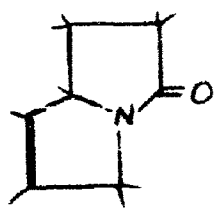
Further improvement was made in another large batch of Mel-150. Small changes in the procedure were made, including isolation of the final product by molecular distillation.

In addition, an interesting solid side product was isolated and analyzed. Elemental analysis (C,H,N) shows the same empirical formula as (I).

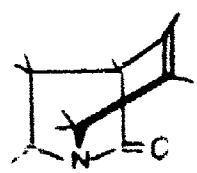
It is very likely that the structure, based on starting materials, is one of the following:



(I)



(II)



(III)

Compound (I) may be ruled out since it is the main product and is an oil. In addition, the IR-spectrum does not show any -C≡CH bond. Structure (II) or (III), is of considerable interest as a possible intermediate for new heterocycles (substitution via C=C-bond). The compound seems to be rather stable. The carbonyl group (of the cyclic amide) could be reduced rather easily with $LiAlH_4$.

It does not appear that the proof of chemical structure would be difficult (ozonolysis, hydrolysis, NMR, hydrogenation). It might be possible to synthesize the compound in question in good yields by simply heating (I), or by an acid-or base-catalyzed reaction of (I).

4.3 Chloroacetylglycylglycine Ethyl ester (MEL-131)[4,5]

Reactions:

$$NH_2 - CH_2 - C(=O) - NH - CH_2 - C(=O) - OH + C_2H_5OH + HCl$$

$$\longrightarrow HCl \cdot NH_2 - CH_2C(=O) - NH - CH_2C(=O) - OC_2H_5 + H_2O \qquad (1)$$

$$HCl \cdot NH_2 - CH_2 - C(=O) - NH - CH_2C(=O) - OC_2H_5 + NaOH \longrightarrow$$

$$NH_2 - CH_2 - C(=O) - NH - CH_2C(=O) - OC_2H_5 + NaCl \qquad (2)$$

$$NH_2 - CH_2 - C(=O) - NHCH_2 - C(=O) - OC_2H_5 + Cl - CH_2 - C(=O) - Cl$$

$$\longrightarrow ClCH_2 - C(=O) - NHCH_2C(=O) - NHCH_2 - C(=O) - OC_2H_5 + HCl \qquad (3)$$

Glycylglycine was esterified with ethyl alcohol in the presence of dry HCl to give the hydrochloride of glycylglycine ethyl ester. The free ester was obtained by treating the hydrochloride with NaOH. The ester decomposes on standing and must be used immediately. Treatment of the ester with chloroacetyl chloride yielded the chloroacetylglycylglycine ethyl ester. A by-product of the last reaction (3) is the hydrochloride of glycylglycine ethyl ester which was separated from the desired product by treatment with hot acetone which dissolved the desired product, but not the hydrochloride.

The melting point of the final product corresponded to that reported in the literature.

4.4 Chloroacetylglycylglycine, Propyl ester (MEL-132)

Reactions:

$$NH_2\text{-}CH_2 - C^{=O} - NH - CH_2\ C^{=O} - OH + C_3H_7OH + HCl \longrightarrow$$

$$HCl \cdot NH_2 - CH_2 - C^{=O} - NH\ CH_2 - C^{=O} - OC_3H_7 + H_2O \qquad (1)$$

$$HCl \cdot NH_2 - CH_2 - C^{=O} - NH\text{-}CH_2 - C^{=O} - OC_3H_7 + NaOH \longrightarrow$$

$$NH_2\text{-}CH_2\text{-}C^{=O} - NH - CH_2 - C^{=O} - OC_3H_7 + NaCl \qquad (2)$$

$$NH_2 - CH_2\ C^{=O} - NH - CH_2 - C^{=O} - OC_3H_7 + Cl - CH_2\ C^{=O} - Cl \longrightarrow$$

$$Cl - CH_2\text{-}C^{=O} - NH - CH_2\text{-}C^{=O}\text{-}NH - CH_2 - C^{=O} - OC_3H_7 + HCl \qquad (3)$$

Glycylglycine was esterified with n-propyl alcohol in the presence of dry HCl to give the hydrochloride of glycylglycine propyl ester. The free ester was obtained by treating the hydrochloride with NaOH. Treatment of the ester with chloroacetyl chloride yielded the chloracetylglycylglycine propyl ester. A by-product of the last reaction (3) is the hydrochloride of glycylglycine propyl ester which was separated from the desired product by treatment with hot acetone which dissolved the desired product, but not the hydrochloride.

The final product was characterized by elemental analysis for carbon, hydrogen, chlorine, and nitrogen.

4.5 Chloroacetyl-Glycylglycine Methyl Ester (MEL-130)

Reaction:

$$ClCH_2C(=O) - NHCH_2C(=O) - NHCH_2 - COOH + CH_2N_2 \longrightarrow$$

$$ClCH_2C(=O) - NH-CH_2C(=O) - NHCH_2C(=O) - OCH_3 + N_2$$

The desired compound was obtained by treatment of chloroacetylglycylglycine with diazomethane in ether.

Elemental analysis for carbon, hydrogen, chlorine, and nitrogen was used to characterize the final product.

4.6 α-Chlorobenzaldoxime (MEL-210)[6]

Reaction:

$$C_6H_5CH = NOH + Cl_2 \longrightarrow C_6H_5\underset{\underset{Cl}{|}}{C} = N-OH + HCl$$

Dry chlorine gas was bubbled into a chloroform solution of benzaldoxime at 5°-10°C. The solvent was evaporated off, and the crude product was recrystallized from petroleum ether to give the desired compound.

The melting point of the final product corresponded to that reported in the literature.

4.7 Preparation of 1,3 Bis-(Triethylammonium) Propane Dibromide (MEL-171)

Reaction:

$$Br-CH_2-CH_2-CH_2-Br + 2(C_2H_5)_3N \xrightarrow[\text{Reflux}]{\text{Methanol}} \left(Et_3\overset{+}{N}-CH_2CH_2CH_2\overset{+}{N}Et_3\right) \cdot 2Br^-$$

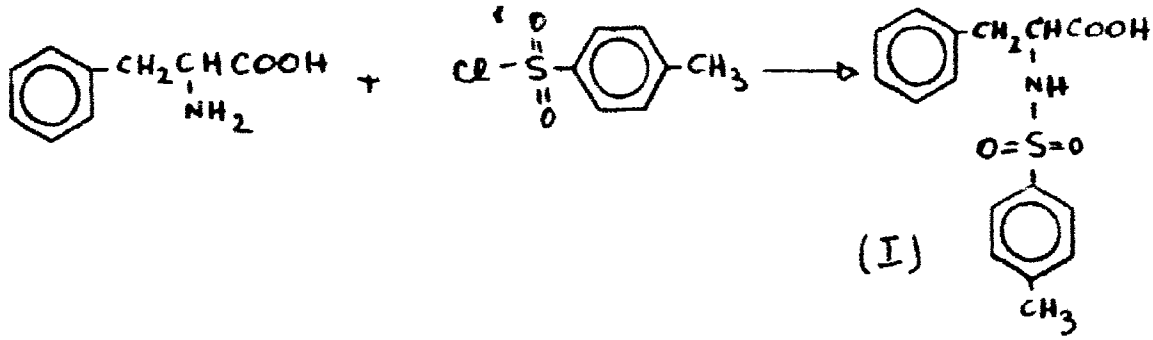
The compound was prepared by refluxing a methanolic solution of 1,3-dibromopropane and excess triethylamine for 72 hours. The yield (31.0%) was fairly low for a quaternization reaction, probably a result of the bifunctionality of the alkyl halide. Longer reaction periods or increased temperatures

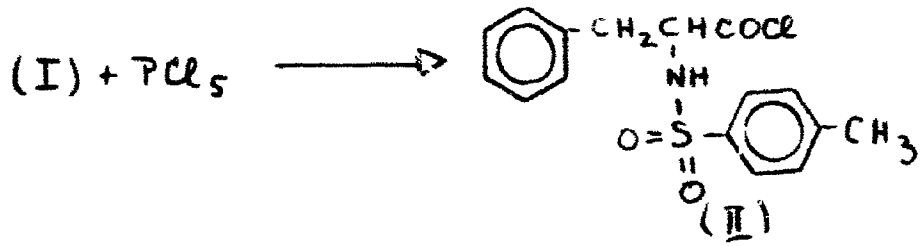
by means of a sealed-tube reaction would probably lead to a significant increase in yield.

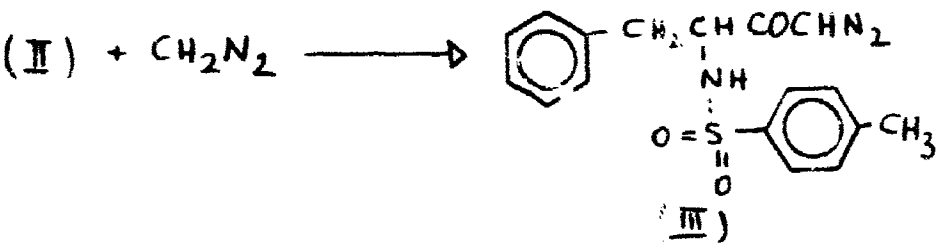
The melting point of the product corresponded to that reported in the literature, and the analysis for Br checked the calculated value.

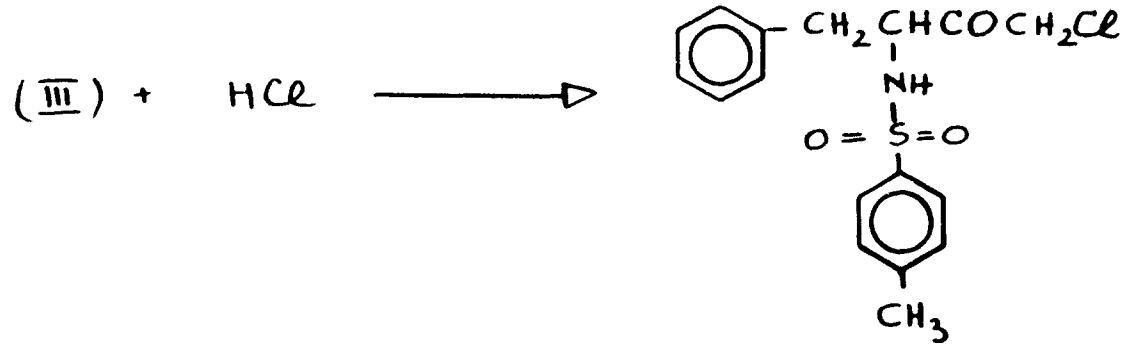
4.8 Preparation of TPCK, 1-1-Tosylamido-2-Phenylethyl Chloromethyl Ketone (MEL-180)

Reactions:









l-Phenylalanine was tosylated with p-toluene sulfonyl chloride in ether solution in the presence of NaOH. After acidification, the crystalline product was recovered and purified by recrystallization.

The tosylated ℓ -phenylalanine was converted to the acid chloride by treatment with PCl_5 in ether.

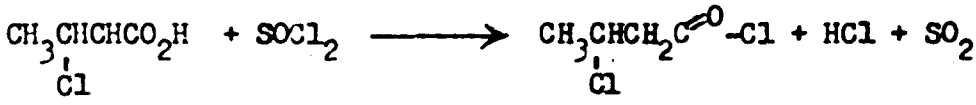
N-tosyl-l-phenylalanyl chloride was then treated with diazomethane in ether to yield the diazomethyl ketone, which was then converted to the desired 1-l-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) by treatment with dry HCl. The product, a white crystalline solid, had a melting point corresponding to that reported in the literature for TPCK.

4.9 Preparation of 4-Chlorobutyryl Chloride (MEL-282)[15]

This compound was commercially available and was procured from Aldrich Chemical Company. Its physical constants (boiling point and refractive index) were checked and compared with those reported in the literature.

4.10 Preparation of 3-Chlorobutyryl Chloride (MEL-283)[16,17]

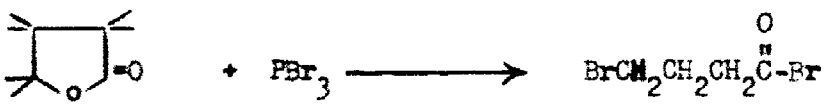
Reaction:



3-Chlorobutyric acid was added dropwise to thionyl chloride while gently warming and stirring the reaction mixture for a period of 30 to 40 minutes. After an additional one-half-hour period of heating at steam bath temperature, the compound was recovered by vacuum distillation. The boiling point and refractive index corresponded to that reported in the literature for 3-chlorobutyryl chloride.

4.11 Preparation of 4-Bromobutyryl Bromide (MEL-281)

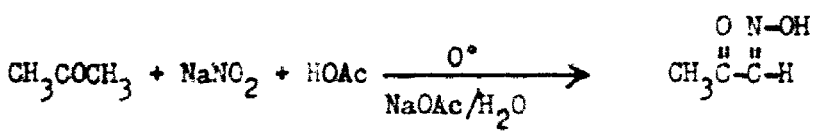
Reaction:



Butyrolactone was heated with phosphorus tribromide for 2 hours at steam bath temperature, then slowly brought up to a temperature of 180°C using an oil bath. The crude oily product was isolated by distillation under reduced pressure. Elementary analysis and comparison of its boiling point with that reported in the literature were used to confirm the identity of 4-bromobutyryl bromide.

4.12 Preparation of Isonitrosoacetone (MEL-231)[19,20,21,22]

Proposed Reaction Route:



A concentrated aqueous solution of sodium nitrite was added dropwise to a cold (0°C) solution of acetone in glacial acetic acid. Water was added, and the mixture was extracted with ether. After drying and removal of the ether, a viscous syrup remained instead of the crystalline mush as

reported in the literature. Attempts to isolate the isonitrosoacetone from the reaction mixture were unsuccessful. Accordingly, the compound was prepared by the following alternate route:

Reactions:

$$CH_3COCH_2COOC_2H_5 \xrightarrow{KOH} CH_3C(=CK)CHCOOC_2H_5 \quad (1)$$

$$CH_3C(=CK)CHC(=O)OEt + NaNO_2 + H_2SO_4 \longrightarrow CH_3C(=O)C(=NOH)COOC_2H_5 + KHSO_4 \quad (2)$$

$$CH_3C(=O)C(=NOH)COOC_2H_5 + NaOH \longrightarrow CH_3C(=O)C(=NOH)COONa + C_2H_5OH \quad (3)$$

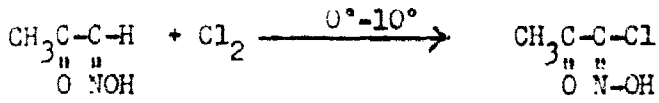
$$CH_3C(=O)C(=NOH)COONa + H_2SO_4 \longrightarrow CH_3C(=O)C(=NOH)COOH \quad (4)$$

$$CH_3C(=O)C(=NOH)COOH \xrightarrow{\Delta} CH_3C(=O)C(=NOH)H \quad (5)$$

A mixture of ethyl acetoacetate and aqueous KOH was allowed to stand at room temperature for 18 hours. A solution of sodium nitrite was then added, and the reaction maintained at 5° to 6°C while dilute sulfuric acid was added dropwise. After 15 minutes, the reaction mixture was neutralized with strong NaOH, then reacidified to pH 4, while keeping the solution cold. Decarboxylation was effected by allowing the reaction mixture to warm to room temperature. The product was extracted with ether. After drying and removal of the ether, the product was recrystallized from benzene. The melting point corresponded to that reported in the literature.

4.13 Preparation of α-Chloro-α-Isonitrosoacetone (MEL-230)[23,24]

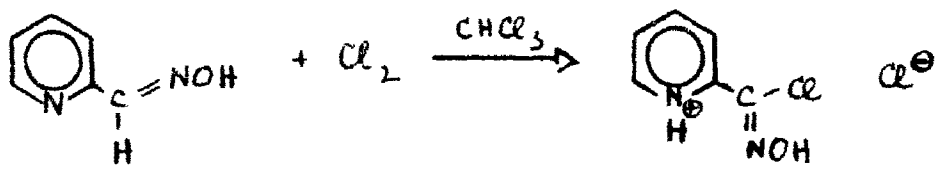
Reaction:



A solution of chlorine and chloroform was added dropwise to a chloroform solution of isonitrosoacetone while maintaining the temperature at 0° to 10°C. After stirring for 1 hour, the chloroform solution containing the final product was evaporated to dryness, and the crude product was recrystallized twice from benzene. The melting point corresponded to that reported in the literature for α-chloro-α-nitrosoacetone.

4.14 Preparation of 2-(α-Chloro)Pyridinealdoxime Hydrochloride (MEL-220)

Reaction:



A solution of 2-pyridinealdoxime in chloroform was cooled to -55°C and chlorine in chloroform solution was added dropwise while stirring the reaction mixture vigorously. The reaction mixture was allowed to warm to room temperature, and the white solid, which had precipitated during reaction, was filtered and dried under vacuum. Elementary analysis for C, H, Cl, and N corresponded to that calculated for 2-(α-chloro)-pyridinealdoxime hydrochloride.

4.15 Preparation of 4-Trimethylammonium Methyl Butyrate Bromide (MEL-174)[25]

Several attempts were made to prepare the above compound before a satisfactory procedure was developed. In the first attempts, the following reactions were investigated.

Proposed:

$$Cl\text{-}(CH_2)_3\overset{O}{\overset{\|}{C}}\text{-}OCH_3 + (CH_3)_2NH \longrightarrow (CH_3)_2N(CH_2)_3\overset{O}{\overset{\|}{C}}\text{-}OCH_3 \qquad (1)$$

$$(CH_3)_2N\text{-}(CH_2)_3\overset{O}{\overset{\|}{C}}\text{-}OCH_3 + CH_3Br \longrightarrow (CH_3)_3N^{\oplus}(CH_2)_3\overset{O}{\overset{\|}{C}}\text{-}OCH_3\ Br^{\ominus} \qquad (2)$$

Treatment of methyl-4-chlorobutyrate with excess dimethylamine at 100°C in a sealed tube gave a colorless oil. Elementary analysis of the oil did not correspond to the calculated values for the desired methyl-4-N,N-dimethylaminobutyrate. Another synthesis route was proposed which was used with success in obtaining the desired compound.

Reactions:

$$BrCH_2CH_2CH_2CN + HBr + 2H_2O \longrightarrow Br\text{-}CH_2CH_2CH_2COOH \qquad (1)$$

$$BrCH_2CH_2CH_2COOH + CH_2N_2 \longrightarrow BrCH_2CH_2CH_2\overset{O}{\overset{\|}{C}}\text{-}OCH_3 \qquad (2)$$

$$BrCH_2CH_2CH_2\overset{O}{\overset{\|}{C}}\text{-}OCH_3 + N(CH_3)_3 \longrightarrow (CH_3)_3\overset{\oplus}{N}CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}\text{-}OCH_3\ \overset{\ominus}{Br} \qquad (3)$$

4-Bromobutyronitrile was hydrolyzed with HBr by refluxing for 2-1/2 hours. The excess HBr was evaporated under vacuum, and the precipitated NH_4Br was filtered and washed with CCl_4. The filtrate was then extracted with CCl_4. After removal of the CCl_4, the 4-bromobutyric acid was isolated as a crystalline solid.

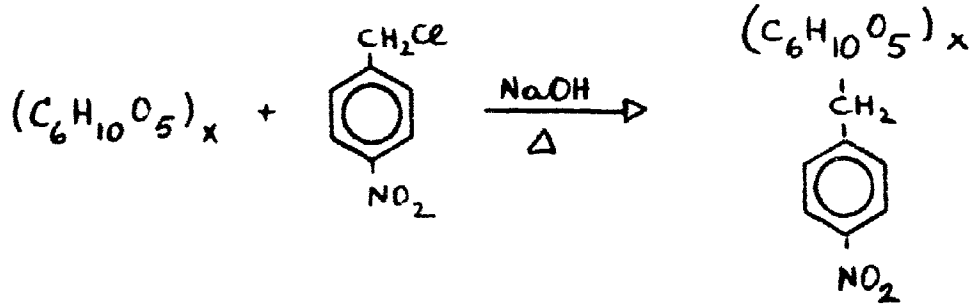
The methyl ester of the 4-bromobutyric acid was prepared using diazomethane.

To prepare 4-trimethyl ammonium methyl butyrate bromide, 4-bromomethyl butyrate was heated with trimethylamine in methanol in an autoclave at 70°C for 24 hours. The methanol was evaporated, and the white solid, which crystallized, was isolated. Purification was effected by dissolving the solid in a minimum amount of methanol and precipitating the product with ether-acetone (6:1). The melting point of the final product did not correspond to that reported in the literature, but elementary analyses for C, H, and Br were very close to the calculated values for 4-trimethylammonium methyl butyrate bromide.

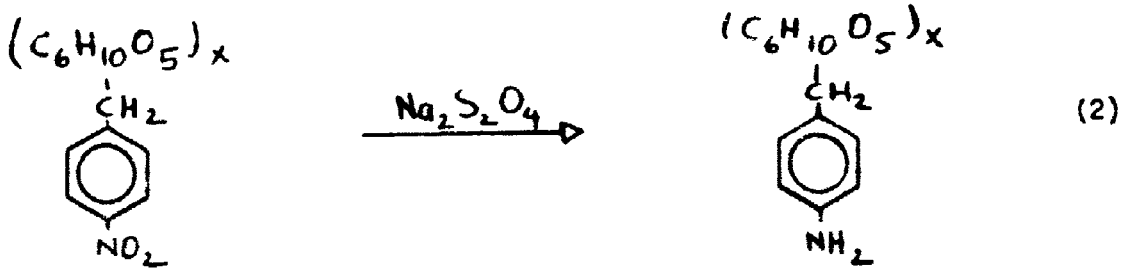
We believe that either the melting point in the literature is not correct or that the previous investigators obtained, not the pure methyl ester, but the ethyl ester arising from transesterification with the solvent ethyl alchol (which they used) or a mixture of methyl and ethyl esters. In addition, the bromine analysis reported in the literature (C and H analyses were not given) corresponds more closely to the ethyl ester than to the methyl ester.

4.16 Preparation of p-Aminobenzyl Cellulose (MEL-320)[27]

Reactions:



(1)



(2)

Powered cellulose, p-nitrobenzyl chloride, and concentrated NaOH were heated at 95°C with vigorous stirring for 4 hours. The mixture was poured into a large excess of water, then filtered and washed thoroughly with water, ethanol, and finally with acetone. The product after drying was the orange-yellow p-nitrobenzyl cellulose.

The p-nitrobenzyl cellulose was suspended in alcohol and reduced to p-animobenzyl cellulose by adding an aqueous solution of sodium hydrosulfite, stirring, and heating at near reflux temperature for 30 minutes. The yellow product was filtered off, washed thoroughly with water, and dried. A sample of the material prepared by the above procedure was submitted to CRDL. Our analysis indicated an exchange capacity of 0.45 meq/g determined by stirring a weighed quantity of the material with an excess of standard HCl, and back titrating with standard NaOH. The validity of the results of this analysis was questionable, however, since it was necessary to stir the material overnight before any significant consumption of HCl was indicated. The same sample, analyzed at CRDL by titrating with an acidic solution of $NaNO_2$ (in order to diazotize the amino groups), indicated that there were practically no amino groups present in the sample.

Three more small development runs were made in which slight changes in procedure and technique were employed in order to obtain a material with a higher ion exchange capacity.

In preparing Sample A (2813-38), a modification in the procedure for isolating the p-nitrobenzyl cellulose consisted of neutralizing the excess NaOH with HCl before the first filtration. This modification greatly reduced the mechanical difficulties originally encountered in filtering the strongly alkaline suspension of p-nitrobenzyl cellulose. No changes were made in the original procedure for reducing the nitro compound. The ion exchange capacity of this sample as determined by the method recommended by CRDL, i.e., titration with acidic $NaNO_2$, was 0.35 meq/g.

In the first step of the synthesis of Sample B (2813-39), the concentration of NaOH was reduced, and the heating time for the reaction of cellulose and p-nitrobenzyl chloride was considerably reduced. The same modification as described above for Sample A was used in isolating the p-nitrobenzyl cellulose. In addition, during the reduction step, the p-nitrobenzyl cellulose was suspended in water instead of alcohol. This seemed to be a better procedure since the reducing agent (sodium hydrosulfite) is water soluble and precipitates out if alcohol is added. The ion exchange capacity of this sample was 0.55 meq/g.

The original procedure was repeated without modification in preparing Sample C. The ion exchange capacity of this sample was 0.40 meq/g.

Since the method of analysis appeared to be subject to variations in technique, all three development samples were submitted to CRDL for confirma- of our analyses.

At a later date, 50 g of p-aminobenzyl cellulose was prepared using the procedure described for preparing Sample B.

4.17 Preparation of 5-Bromovaleryl Bromide (MEL-284);[28,29]

Reactions:

$$BrCH_2CH_2CH_2CH_2CN + HBr + H_2O \xrightarrow{\Delta} BrCH_2CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}-OH + NH_4Br \quad (1)$$

$$BrCH_2CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}-OH + SOCl_2 \longrightarrow BrCH_2CH_2CH_2CH_2C(=O)-Cl + SO_2 + HCl \quad (2)$$

$$BrCH_2CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}-Cl + HBr \longrightarrow BrCH_2CH_2CH_2CH_2C(=O)Br + HCl \quad (3)$$

A mixture of 5-bromovaleronitrile and 48% HBr was refluxed 2-1/2 hours with stirring. The precipitated NH_4Br was filtered off and the product extracted with CCl_4. After removal of solvent at reduced pressure, crystalline 5-bromovaleric acid having a melting point corresponding to that reported in the literature was obtained.

The acid obtained above was heated at reflux temperature with thionyl chloride for 2 hours. Distillation of the reaction mixture at reduced pressure yielded a colorless liquid with a boiling point corresponding to that reported in the literature for 5-bromovaleryl chloride.

Dry HBr was bubbled into cold 5-bromovaleryl chloride for 3 hours. Distillation of the reaction mixture afforded a product boiling at 75°-76° at < 1-mm pressure. Elementary analysis for C, H, and Br corresponded reasonably well to that calculate for 5-bromovaleryl bromide.

4.18 Preparation of 3-Bromopropionyl Bromide (MEL-285)[29]

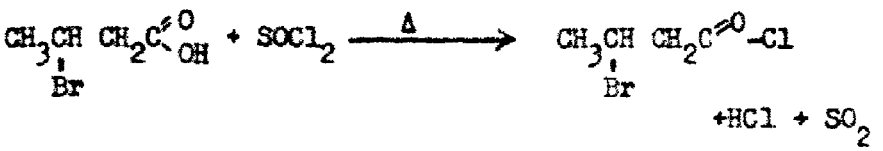
Reaction:

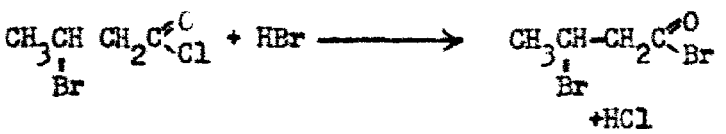
$$BrCH_2CH_2C(=O)Cl + HBr \longrightarrow BrCH_2CH_2C(=O)Br + HCl$$

Dry HBr was bubbled into cold 3-bromopropionyl chloride for 3 hours. Fractional distillation of the reaction mixture at 20 mm afforded the 3-bromopropionyl bromide boiling at 79°-84°C. Elementary analysis for C, H, and Br confirmed the identity of the product.

4.19 Preparation of 3-Bromobutyryl Bromide (MEL-280)[29]

Reactions:



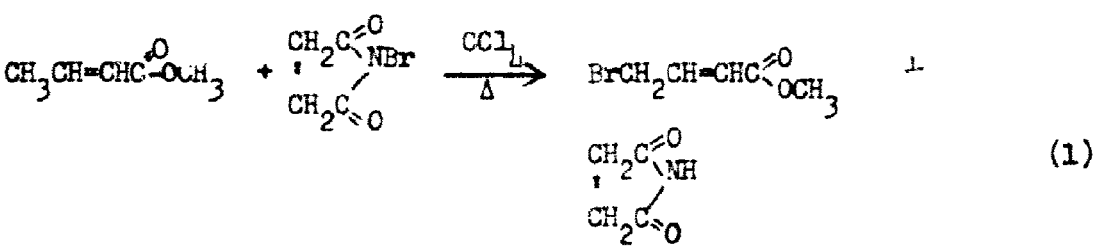


3-bromobutyric acid was heated at reflux temperature with thionyl chloride for 2 hours. The reaction mixture was distilled at reduced pressure to obtain the acid chloride.

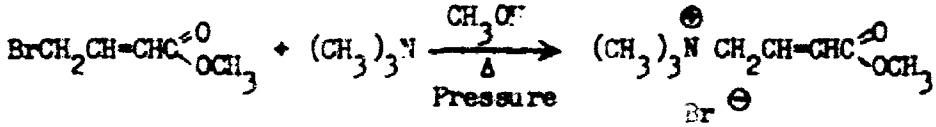
Dry hydrogen bromide was bubbled into cold 3-bromobutyryl chloride for 3 hours. Distillation of the reaction mixture yielded the product boiling at 50°-60° at 3-mmHg pressure. Elementary analysis for C, H, and Br corresponded reasonably close to that of 3-bromobutyryl bromide.

4.20 Preparation of 4-Trimethylammonium Methyl Crotonate Bromide (MEL-172)[30]

Reactions:



(1)



(2)

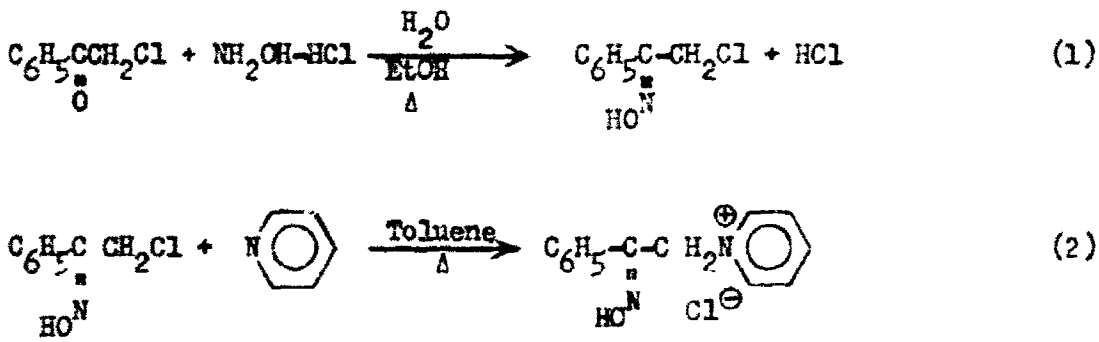
Methyl crotonate was added to a suspension of N-bromosuccinimide in CCl_4. The mixture was stirred and refluxed for 12 hours. The succinimide which formed was filtered off and the solvent removed by distillation. The product, isolated by distillation at reduced pressure, had a boiling point corresponding to that reported in the literature for methyl-4-bromocrotonate.

In the first attempt at quaternization, the reaction mixture was heated in an autoclave at about 70°C for 24 hours. Extensive polymerization appeared to occur, and the low yield of crystalline material which was isolated did not give the correct elementary analysis for 4-trimethylammonium methyl crotonate bromide.

The experiment was repeated with a modification in the quaternization step. Instead of heating in an autoclave at 70°C., the reaction mixture was simply rocked in an autoclave for 2 days at room temperature. The yield of crystalline material from this run was much greater than in the first experiment, and the amount of polymeric tars appeared to be negligible. The compound, purified by precipitation from a concentrated methanol solution with acetone-ether, melted at 153°-6°C. Elementary analysis for C and H corresponded to that of 4-trimethylammonium methyl crotonate bromide.

4.21 Preparation of α-Pyridinium Acetophenone Oxime Chloride (MEL-250)[31]

Reactions:



(1)

(2)

A solution of α-chloroacetophenone in ethanol was combined with an aqueous solution of hydroxylamine hydrochloride and the mixture was warmed until all solids were dissolved. Upon cooling and addition of more water, the oxime precipitated and was purified by recrystallization from carbon disulfide.

Pyridine was added to a cold solution of chloroacetophenone oxime in toluene. Some solid product precipitated immediately, and heating the reaction mixture caused more product to come out of solution. The white solid was filtered off and purified by dissolving in methanol and precipitating with anhydrous ethyl ether. Elementary analysis for C, H, N, and Cl corresponded reasonably well with the calculated values for α-pyridinium acetophenone oxime chloride.

4.22 Preparation of 4-Dimethyl Sulfonium Methyl Butyrate Iodide (MEL-175)[32, 33]

Reactions:

$$Na + CH_3SH \xrightarrow{MeOH} NaSCH_3 \qquad (1)$$

$$NaSCH_3 + BrCH_2CH_2CH_2C(=O)OCH_3 \longrightarrow CH_3SCH_2CH_2CH_2C(=O)OCH_3 + Na\,Br \qquad (2)$$

$$CH_3SCH_2CH_2CH_2C(=O)OCH_3 + CH_3I \longrightarrow I^{\ominus}\ CH_3\overset{\oplus}{S}(CH_3)(CH_2CH_2CH_2C(=O)OCH_3) \qquad (3)$$

Several previous attempts to prepare 4-dimethyl sulfonium methyl butyrate bromide by reacting methyl 4-bromobutyrate and dimethyl sulfide were unsuccessful. In these experiments, the only product isolated appeared to be trimethyl sulfonium bromide. Therefore, preparation of the compound by an alternate route was attempted.

In the first attempt to prepare sodium methyl mercaptide, sodium and methyl mercaptan were allowed to react in liquid ammonia. The product obtained by this procedure analyzed only 72% pure. In another procedure, sodium methyl mercaptide was prepared by reacting sodium with methyl mercaptan in methanol. After addition of toluene to the reaction mixture, the resulting sodium methyl mercaptide was filtered and dried. Analysis of the salt by oxidation with I_2 and back titration with $Na_2S_2O_3$ indicated a purity of practically 100%.

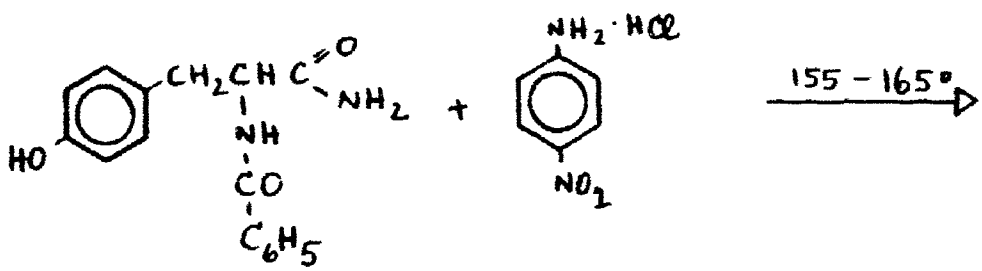
The thioether, 4-(methyl butyrate) methyl sulfide, was prepared by refluxing methyl 4-bromobutyrate and sodium methyl mercaptide (100% purity) in methanol for 7 hours. After evaporation of the solvent, the residue was taken up with water and extracted with ether. The ether was removed and the thioether purified by vacuum distillation (B.P. 91°-92°/12mm).

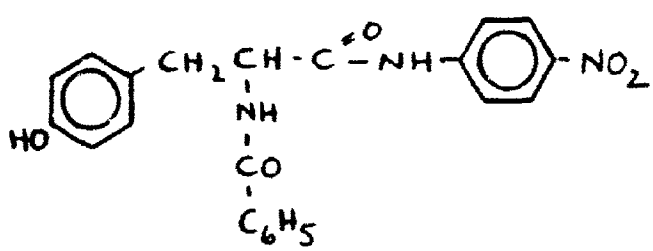
A previous attempt using the 72% pure sodium methyl mercaptide gave a poor yield of impure thioether which was discarded.

The final sulfonium salt was prepared by reacting an acetone solution of the thioether described above with methyl iodide for 2-1/2 hours at room temperature. Upon addition of ether, white crystals precipitated and were filtered off. Purification was effected by redissolving the compound in acetone and precipitating with ether. Elementary analysis for C and H checked the calculated values for 4-dimethyl sulfonium methyl butyrate iodide.

4.23 Preparation of N-Benzoyl-l-Tyrosine-p-Nitroanilide (MFL-290)

Reaction:



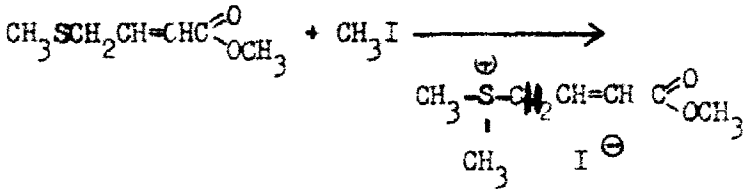


p-Nitroaniline was reacted with gaseous HCl in benzene to give the corresponding hydrochloride which subsequently was mixed with N-benzoyl-l-tyrosine amide and heated to 155°-165° for 3/4 of an hour. The light brown product was purified by repeated precipitation from ethanol with water and finally with n-hexane. The melting point (225°-8°) corresponded to that reported in the literature.

4.24 Preparation of 4-Dimethyl Sulfonium Methyl Crotonate Iodide (MEL-173)

Reactions:

(1)



(2)

4-(methyl crotonate) methyl sulfide was prepared by refluxing a methanolic solution of methyl-4-bromocrotonate and sodium methyl mercaptide for 7 hours. After evaporation of the solvent, the residue was taken up with water and extracted with ether. The ether was removed and the thioether purified by distillation under vacuum.

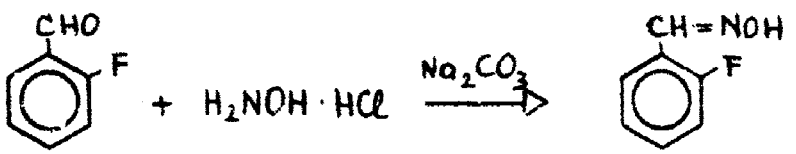
The sulfonium iodide was prepared by reacting the thioether described above with methyl iodide in acetone solution. After standing for 16 hours at room temperature, a trace of crystalline material precipitated (m.p. 214°-215°). This material is probably trimethyl sulfonium iodide. The main yield of material was recovered as an oil after removal of the acetone solvent under reduced pressure. The oil failed to crystallize in spite of

extensive effort and employment of a number of techniques. The solubility properties of the oil (soluble in water, EtOH, MeOH, $CHCl_3$; insoluble in ether, hexane, CCl_4, and benzene) suggest that it is the impure sulfonium salt. To further purify the substance, it was dissolved in water, extracted with ether, and the aqueous layer was evaporated under vacuum at 35°. The resulting oil was dried further under vacuum over P_2O_5. The elementary analysis for C and H corresponded to the calculated values for 4-dimethyl sulfonium crotonate iodide.

It is possible that the oil may be a salt mixture of the cis and trans crotonate, which would account for a depression in the melting point, and might explain the difficulty in obtaining a crystalline salt.

4.25 Preparation of 2-Fluorobenzaldoxime (MEL-222)[35]

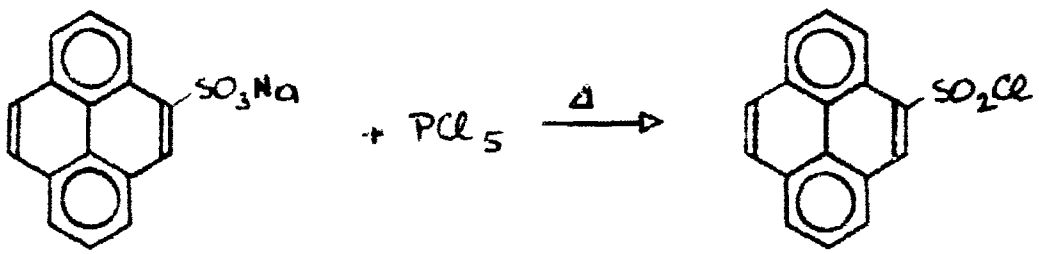
Reaction:



2-Fluorobenzaldehyde and hydroxylamine hydrochloride were reacted in the presence of sodium carbonate in a water-ethanol mixture. The oxime which precipitated was recrystallized from hexane. The melting point corresponded to that reported for 2-fluorobenzaldoxime.

4.26 Preparation of Pyrene-3-Sulfonyl Chloride (MEL-121)[36, 37]

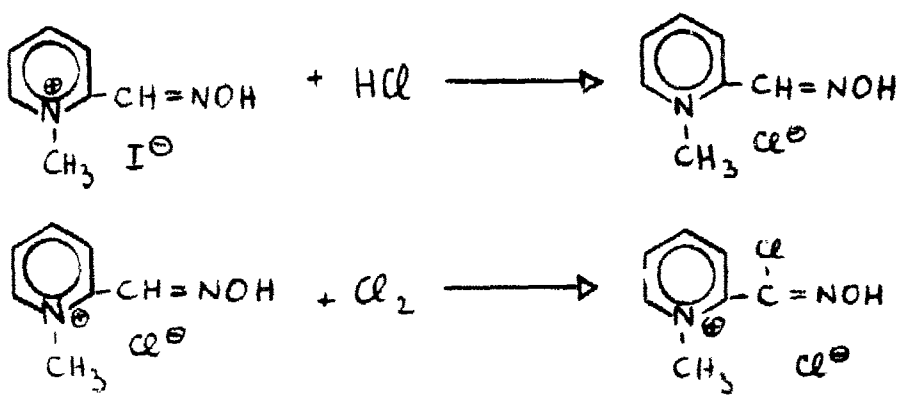
Reaction:



After several unsuccessful attempts to prepare the compound by the above reaction, a technique was developed which yielded the pyrene-3-sulfonyl chloride with an elementary analysis for chlorine corresponding to the theoretical value. The sodium salt of pyrene-3-sulfonic acid was heated slowly with an equimolar amount of PCl_5 until the temperature reached 155°. The reaction mixture was held at this temperature for 15 hours, then cooled and treated with ice water. The yellow solid remaining was filtered and dried under vacuum, then treated again with ice water, filtered, and dried under vacuum. The solid was dissolved in chloroform, precipitated with hexane, filtered, and dried.

4.27 Preparation of α-Chloro-2-Pyridine Aldoxime Methchloride (MEL 221)[27]

Reactions:



In many previous attempts to prepare the above compound, 2-pyridine aldoxime methiodide was reacted directly with chlorine under a variety of conditions. In none of these experiments was it possible to isolate the final desired product of satisfactory purity.

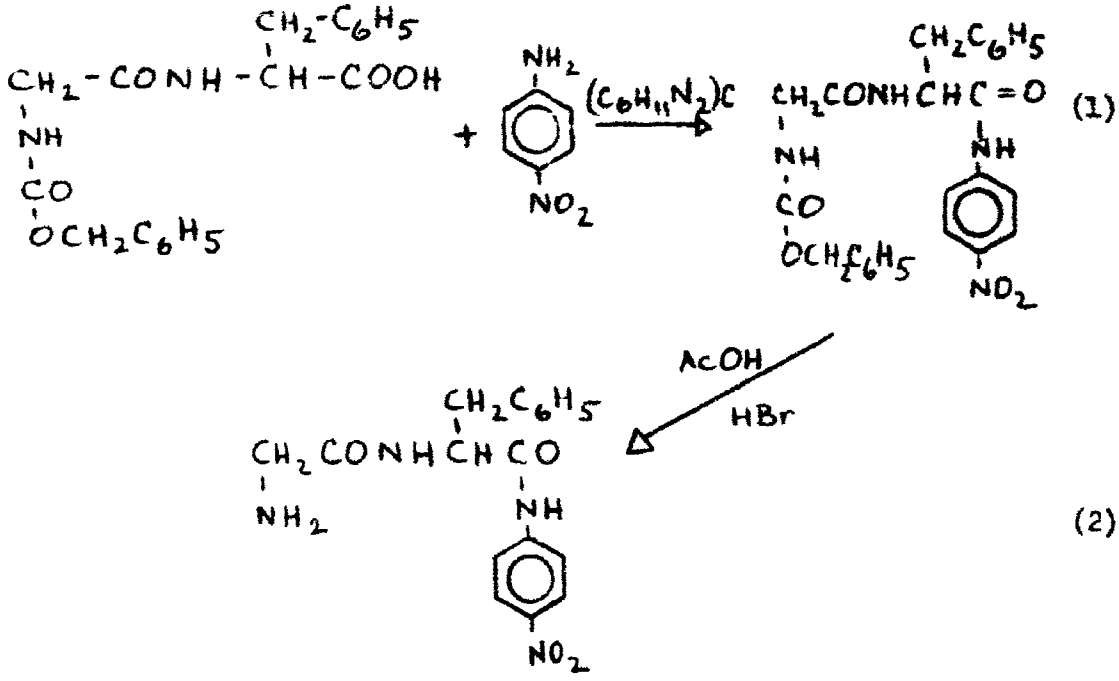
Finally, a two-step synthesis as shown above was employed. The first preparation using this approach yielded a product slightly contaminated (ca. 5%) with NaCl. By slightly modifying the work-up procedure in a subsequent preparation, we obtained the pure α-chloro-2-pyridine aldoxime methchloride. The infrared spectrum of the pure compound and that of the product slightly contaminated with NaCl were identical, indicating that NaCl in the latter was the only significant impurity.

2-pyridine aldoxime methiodide was converted to the corresponding methchloride by reacting it with dry HCl in methanol.[41] The 2-pyridine aldoxime methchloride was isolated by precipitation with a 50-50 mixture of ether and acetone.

To the 2-pyridine aldoxime methchloride in methanol at 0°C was added simultaneously a solution of Cl_2 in methanol and a solution of sodium methoxide in methanol. The reaction mixture was allowed to stand over night at room temperature, and the NaCl which formed was filtered off. Elementary analysis of the product recovered from the filtrate corresponded with the calculated values for α-chloro-2-pyridine aldoxime methchloride.

4.28 Preparation of Glycylphenylalanine -p- Nitroanilide (MEL-350)[29, 30]

Reactions:



In one reference[29] it is claimed that the above compound was prepared by a method analogous to the preparation of lysine-p-nitroanilide which was described elsewhere.[30] The yield was stated to be 4%, but no details were given for the preparation nor do the authors give any analysis, melting point, or other physical constants. We followed the method analogous to the preparation of lysine-p-nitroanalide and isolated a product which proved to be extremely hygroscopic. Strictly anhydrous solvents are required for recrystallization.

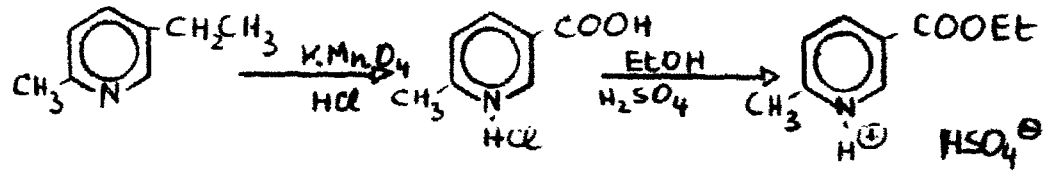
In a second batch the yield was remarkably increased (to 96%). Since the compound is so hygroscopic that even standing in open air for less than a minute gives an oil. Strictly anhydrous conditions in all operations are required; otherwise, the yield decreases remarkedly (4% according to the literature cited).

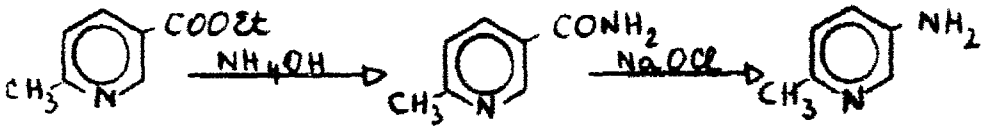
5. SYNTHESIS RESEARCH IN PROGRESS

This part includes incomplete preparations and discussion of parts of the multistep syntheses. It also includes discussion of synthesis of starting materials for compounds not delivered and discussion of unsuccessful preparations.

5.1 Methyl-5-Amino Pyridine (MEL-1141)[7,8,9]

Reactions:





2-Methyl-5 ethylpyridine was selectively oxidized with aqueous potassium permanganate and isolated as the copper complex. The oxidation and work-up of the 6-methyl nicotinic acid was a slow, tedious step requiring concentration of the product from large volumes of water. The copper complex was dissolved in hydrochloric acid and freed from copper by treating with H_2S and the 6-methyl-nictotinic acid hydrochloride isolated.

Esterification of 6-methyl-nicotinic acid with ethyl alcohol in the presence of H_2SO_4 yielded ethyl 6-methyl nicotinate which was isolated by vacuum distillation.

The ester was converted to 6-methyl nicotinamide by stirring overnight with ammonium hydroxide, filtering, and drying.

Treatment of the amide with sodium hydrochlorite (Chlorox) in the presence of KOH yielded by Hoffmann degradation the desired 2-methyl-5 amino pyridine.

The melting point of the final product, as well as the melting or boiling points of all the intermediates isolated, corresponded to those reported in the literature.

A somewhat more convenient route to 6-methyl nicotinic acid was checked out in one run. This involved selective oxidation of 2-methyl-5-ethyl pyridine with concentrated HNO_3 in the presence of a catalytic amount of ammonium vanadate. The yield was about the same as with the permanganate, but the work-up was a little more convenient.

5.2 Attempted Preparation of α -Bromobenzaldoxime (MEL-211)

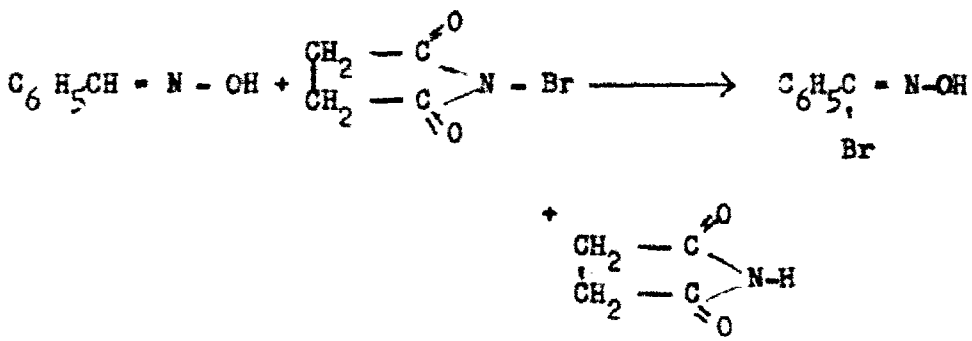
Proposed reaction route:

$$C_6H_5CH = N\text{-}OH + Br_2 \longrightarrow C_6H_5\,\underset{\displaystyle Br}{\overset{\displaystyle |}{C}} = NOH + H\,Br$$

Bromine was added to a cold (5°-10°C) solution of benzaldoxime in chloroform solution. Although some reaction occurred, the products isolated after evaporation of solvent and recrystallization from a variety

of solvents appeared to be mixtures (as judged by wide melting point ranges). Failure to obtain the desired product was probably due to bromination of the ring in one or more positions as significant side reactions, or perhaps the only reactions, under the conditions employed.

A second proposed reaction route is as follows:



A solution of benzaldoxime in chloroform was added to a chloroform suspension of N-bromosuccinimide. Upon heating and stirring, a reaction took place and all solids went into solution. On cooling, succinimide separated out. The succinimide was filtered and the filtrate was concentrated until a product separated on cooling. This product, which was originally thought to be the α -bromobenzaldoxime, proved to be slightly impure succinimide.

Benzaldoxime (12.1 g) was dissolved in 150 ml of dry $CHCl_3$. Sodium methoxide (55 g) was added, and the mixture was placed in a quartz flask fitted with a stirrer and dropping funnel. With stirring and ultraviolet radiation (using a sunlamp) 16.0 g of bromine in 100 ml of $CHCl_3$ was added within 20 minutes. The reaction mixture was filtered to remove the sodium bromide and the mother liquor evaporated under vacuum. The

residual oil crystallized. It was dissolved in 15 ml of $CHCl_3$ and precipitated again with CCl_4 to give yellow-orange crystals which were extremely sensitive to moisture. The yield was 7.5 g.

In a similar experiment using triethanolamine as the HBr acceptor, the yield was much lower, i.e., 3.0 g.

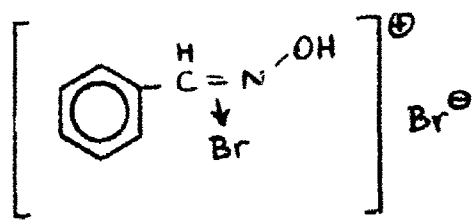
In another experiment using the same amounts of starting material as described above but with the difference of cooling the mixture to -30°C during the reaction, the yield was almost the same as described in the first experiment.

Because of solubility problems, CCl_4 appeared to be the better solvent. The reaction was carried out with the same amounts as described above with the following differences: (1) the reaction mixture was cooled to -30°C and (2) the solvent was CCl_4. The bromine solution and a suspension of MeONa in 100 ml of CCl_4 were added simultaneously at the same rate from two separate dropping funnels. Sodium bromide and the reaction product precipitated during the reaction. The precipitate was filtered, was extracted with $CHCl_3$, and was precipitated with CCl_4 to give 8.0 g of yellow-orange crystals (m.p., 94.0° to 94.5°C.)

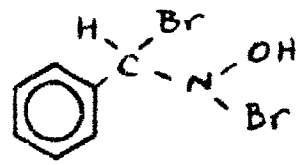
The compound isolated in the last experiment was extremely sensitive to water and even to moist air.

In attempting to characterize the yellow-orange crystals (m.p., 94.0° to 94.5°C) isolated in the last experiment, 0.560 g was reacted with water and titrated with 0.1 N NaOH. It required 36.7 ml of NaOH to neutralize (to the phenolphthalein endpoint) the free acid. This

corresponds to two very labile Br atoms in the molecule. If one Br is on the benzene ring, it would not be expected to hydrolyze so easily. Therefore, it is concluded that the two Br atoms are in the side chain. In the present state of our limited study, we can only vaguely speculate as to the structure of the compound which might be one of the following:



(I)



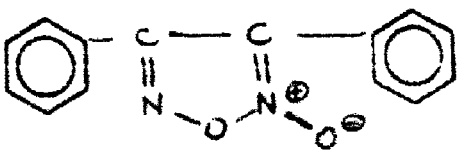
(II)

The insolubility of the compound in CCl_4 suggests that structure (I) is more likely. Elemental analysis:

$C_7H_7Br_2NO$		Calculated	Found
	C	29.2	29.31, 30.01
	H	2.5	2.9 , 2.76
	Br	57.0	58.19

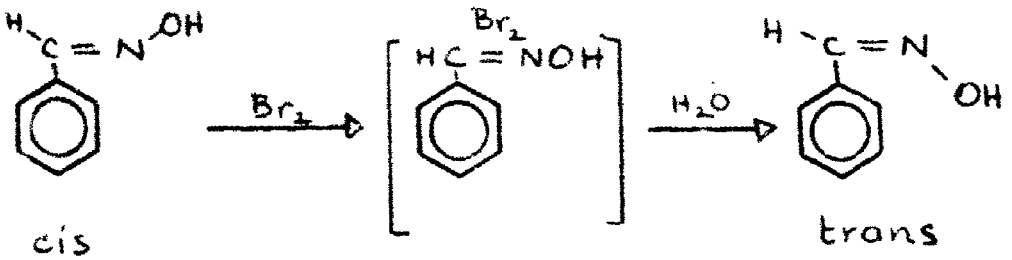
Water hydrolyzed the compound rapidly, and the resulting solution indicated the presence of active bromine (starch-iodide paper turns blue). The white crystals which can be precipitated after hydrolysis, upon purification, had a melting point of 127° and a molecular weight of 137. Furoxanes are usually by-products in the halogenation of aldoximes; but in this case the molecular weight shows that the product is not

diphenylfurexane (III), which also has a melting point close to that of trans benzaldoxime.



(III)

Microanalysis, molecular weight, and melting point show that the compound isolated after hydrolysis is trans benzaldoxime, and the original starting material was cis benzaldoxime:



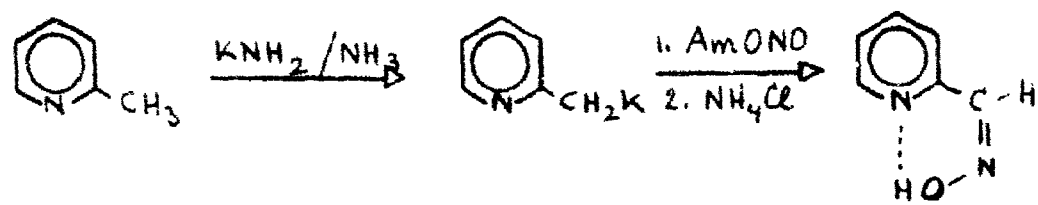
Analysis: C_7H_7NO	Calc.	Found
	C 69.5%	C 69.64%
	H 5.8	H 6.68
	N 11.58	N 11.21
		Br 0.0

Molecular weight: Calc: 121, found: 137.

Further examination of the brominated product by NMR and UV spectroscopy is planned. It is expected that such studies will give an answer as to whether the compound is a charge transfer complex, an adduct of Br_2 with benzaldoxime, a salt such as a hydrobromide, or a covalent compound with one bromide on the α -carbon and the second bromine bound to the nitrogen or oxygen.

5.3 Preparation of Pyridine-2-Aldoxime

Proposed reaction route:

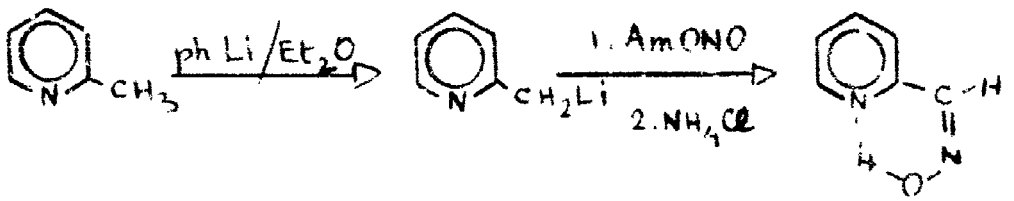


The purpose of this experiment was to check out the above procedure which was recently reported in the literature.[10] If successful, it was planned to make use of the method for making oximes with certain substituted 2-picolines as starting materials.

After preparing potassium amide from metallic potassium and liquid ammonia, 2-picoline was added followed by amyl nitrite. After stirring for 1-1/2 hours, dry ammonium chloride was added, and the excess ammonia was allowed to evaporate. The residue was extracted with ether. After distillation to remove ether, the unreacted picoline was distilled under reduced pressure, and an attempt was made to distill the product from the tarry residue at 1-mm pressure. A trace of semicrystalline material which

appeared to sublime on the neck of the apparatus was recovered, but no positive identification could be made.

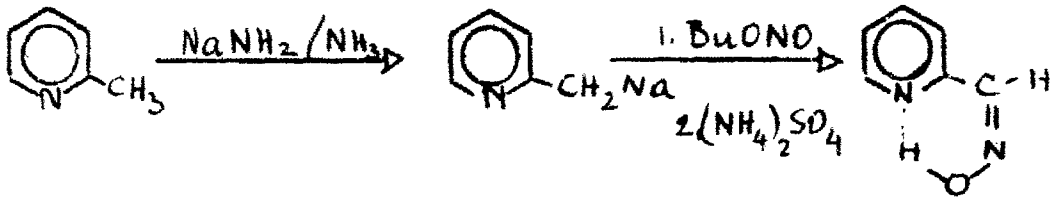
A second proposed procedure is as follows:



2-picoline was added to phenyllithium prepared from metallic lithium d bromobenzene in ether. The lithio-picoline was added to an ether ution of amylnitrite, and the reaction mixture was stirred at room perature for 2 hours. Aqueous ammonium chloride was added, and the r layer separated. The aqueous layer was extracted several times ether, and the washings were combined with the main ether layer. drying, the solution was distilled to remove ether and other low- ng components. Unreacted 2-picoline and an unidentified liquid were d by distillation under reduced pressure. Attempts to recover the product by distillation of the tarry residue at 1-mm pressure successful. There was some evidence that the product might be d by vacuum sublimation, and a small amount of oily solid was in this manner. After trituration of this solid with petroleum recrystallization from benzene, a very small amount of e material with a melting point corresponding to that reported erature for 2-pyridine aldoxime was isolated.

A variation was attempted using (1) a large excess of 2-picoline and amyl nitrite with respect to sodium (sodium in liquid ammonia instead of potassium was used in these experiments) and (2) $(NH_4)_2SO_4$ instead of NH_4Cl. It was not possible to isolate any of the desired 2-pyridine aldoxime in this experiment.

Through improvement in the technique of isolation, substitution of butyl nitrite for amyl nitrite, and variation in the ratio of reactants, it was possible to obtain a respectable yield of 2-pyridine aldoxime via the sodio-picoline route. The successful procedure, similar to that reported by S. Forman,[26] follows in detail:

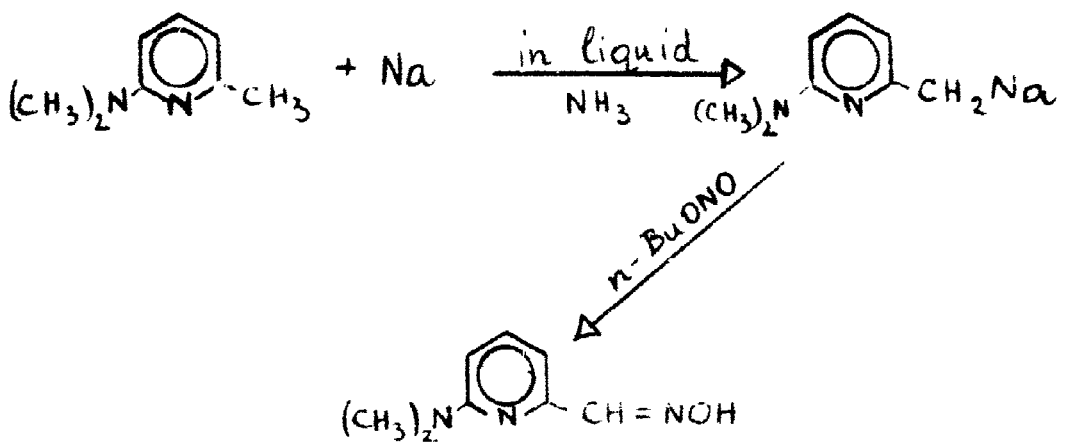


Sodamide was made in the usual manner from sodium metal (23 g, 1g atom) and liquid NH_3 (400 ml). 2-picoline (139.6 g, 1.5 moles) was added dropwise over a period of 1 hour, and the reaction mixture was stirred for 2 hours. Freshly prepared n-butyl nitrite (51.5 g, 9.5 mole) was added dropwise to the reaction mixture over a period of one-half hour, and the mixture was allowed to stir an additional 20 minutes. Unreacted $NaNH_2$ was decomposed by adding dry $(NH_4)_2SO_4$.

The NH_3 was allowed to escape at room temperature while replacing it with 250 ml ether. After all of the NH_3 had escaped, a thick, yellow slurry remained. Saturated aqueous $(NH_4)_2SO_4$ was added until all solids dissolved. The layers were separated, and the ether layer was washed with the saturated $(NH_4)_2SO_4$ solution. The aqueous phase and washings were combined and extracted with ether. The combined ether extracts were dried over $MgSO_4$, then distilled to remove ether. Excess picoline and other volatile components were removed by vacuum distillation up to a pot temperature of 60°C. Finally, there remained a dark yellow solid which was recrystallized from benzene three times to yield a slightly yellow product having a m.p. of 113.5° to 114.5°C (literature m.p. for 2-pyridine aldoxime is 115°C). Further purification to obtain a white product was effected by vacuum sublimation at about 1 mm and 70°C. The m.p. was 115° to 116°C. The yield was 24.1 g or 39.6%, based on butyl nitrite.

5.4 Attempted Preparation of 6-Dimethylamino-Pyridine-2-Aldoxime (MEL-149)

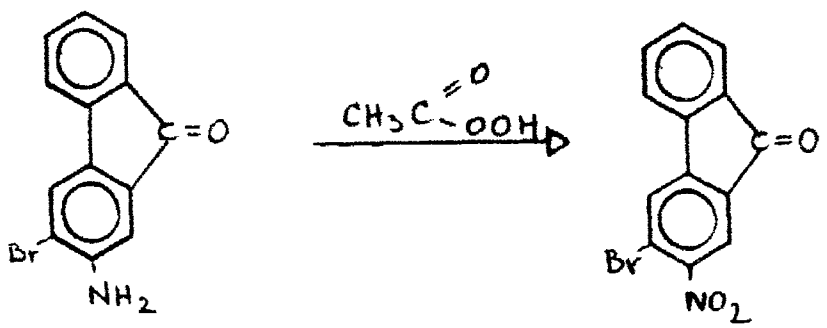
Proposed Reaction Route:



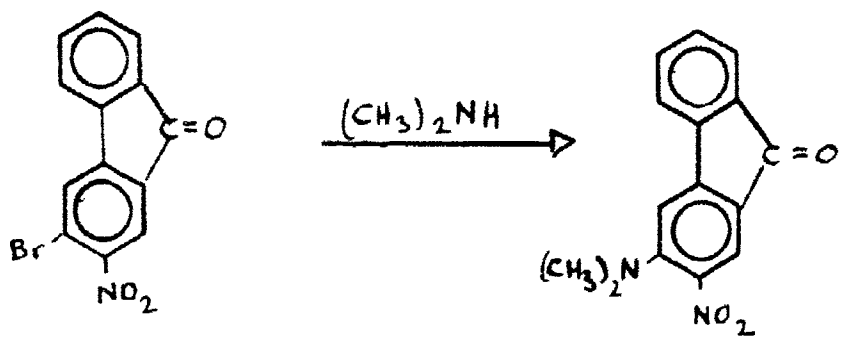
This same procedure (as described under 5.3) was used in this preparation, but the desired compound could not be isolated. After removal of excess 6-dimethylamino 2-picoline, a deep reddish-brown oil remained. A solid consisting of a trace of yellow crystals mixed with a brown, gummy material was precipitated from the reddish-brown oil. Attempts to purify this material by recrystallization and by sublimation were not successful, and only a trace of gummy yellow solid was isolated.

5.5 2-Amino 3-N,N Dimethylamino Fluorenone-9 (MEL 300)[38]

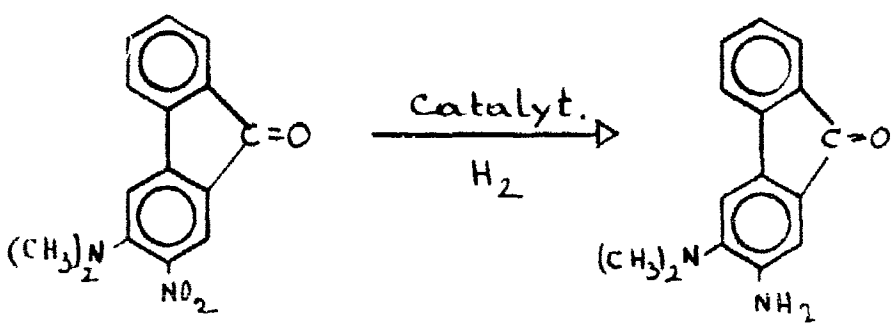
Reactions:



(1)



(2)



(3)

A direct preparation of the 2-amino 3-N, N dimethyl fluorenone-9 by reaction of 2-amino 3-bromo fluorenone-9 with dimethylamine proved to be difficult. This reaction, carried out by heating the reactants in an autoclave at 100° for 2 hours, gave a mixture consisting mostly of starting material and small amounts of unidentified products. It was decided, therefore, to investigate the longer synthesis route shown above, especially since some of the reactions have been described in the literature for making very similar fluorene derivatives.

2-Nitro-3-bromofluorenone-9 was prepared in good yield by oxidation of the corresponding amino compound with 38% peracetic acid.

2-Nitro-3-N, N dimethylamino fluorenone-9 was synthesized by reacting the bromo derivative above with excess dimethylamine in an autoclave at 70° for 2-1/2 days. A mixture of benzene and ethanol was used as a solvent. Shiny, gold-brown crystals melting at 233 -4° precipitated from the reaction mixture. The compound was almost completely insoluble in ethanol, methanol, ethyl acetate, ether hexane, dioxane, and THF. It was slightly soluble in acetic acid, acetone, dimethylformamide, and N-methyl pyrrolidone.

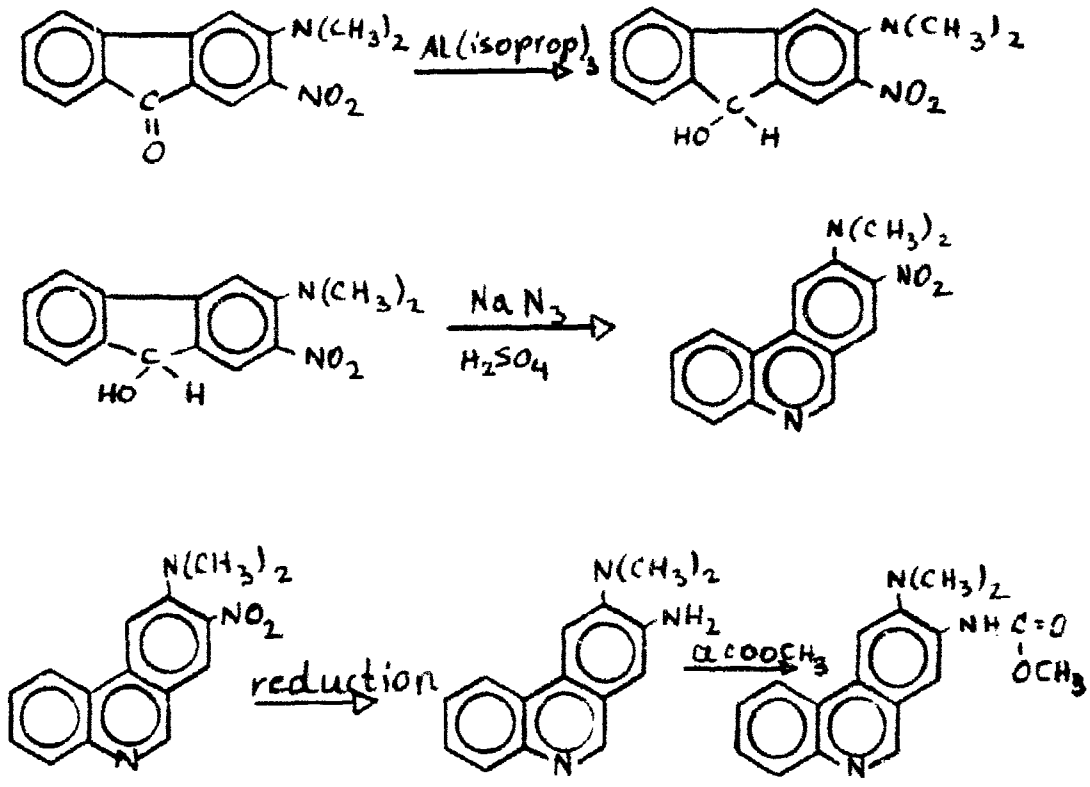
Several attempts were made to convert 2-nitro-3 N, N dimethyl amino fluorenone-9 to the 2-amino compound by catalytic hydrogenation with Pt black. This appears to be difficult because of the low solubility of the nitro compound. With ethanol as a solvent, no hydrogenation was observed. With glacial acetic acid as a solvent, the product proved to be mostly starting material. Using dimethylformamide as a solvent, slow hydrogen uptake was observed. After evaporation of the solvent under vacuum, a dark-red solid crystallized which after washing with water was dissolved

in acetone and p.ecipitated with hexane to give a purple solid melting at 133°-135°. Elementary analysis of this product corresponded to the theoretical values for 2-amino 3-N, N dimethyl fluorenone-9.

Detailed experimental procedures are given in the appendix.

5.6 6-N, N Dimethylamino-7-Methyl Carbamyl Phenanthridine (MEL 263)

Reactions:



It is known that aromatic ketones can be specifically reduced with $NaBH_4$ (at room temperature) to the corresponding alcohol without reducing a nitro group attached to the aromatic ring.

Several attempts were made to reduce the nitro ketone with $NaBH_4$. However, it appeared that the nitro ketone was too insoluble at room temperature (solvents: methanol and isopropanol). The only product isolated at room temperature was starting material. At elevated temperatures reduction of the nitro group as well as the ketone occurred. It was desirable to avoid reduction of the nitro group since the subsequent rearrangement to the phenanthridine gives much poorer yields with the amino compounds. Therefore, reduction of the ketone by the alternate method of Meerwein-Ponndorf with aluminum isopropoxide was attempted. This proved to be a rather clean reaction and gave the alcohol in good yield. Unreacted starting material could be easily recovered at the end of the reaction by filtration.

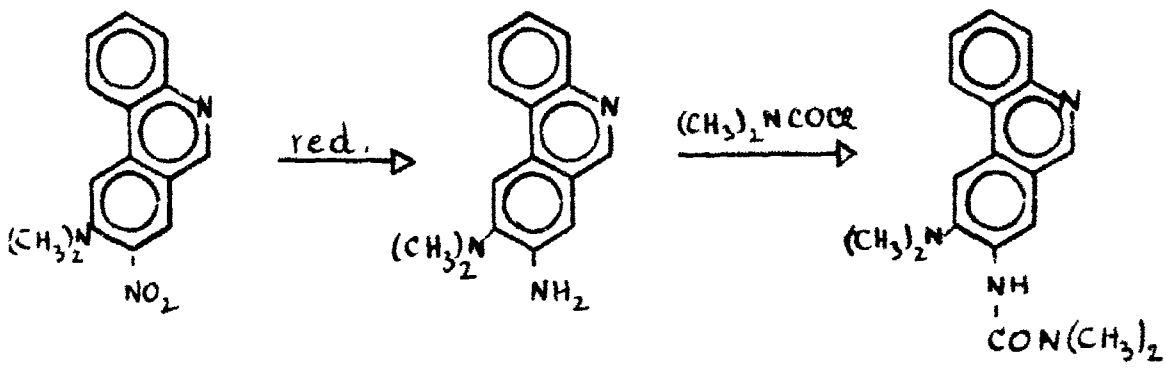
In different experiments we increased the reaction time from 1/2 hour to 2-1/2 hours. The conversion increased from 40% to 69%. The overall yield in each experiment was 95% since unreacted material was recovered.

The rearrangement of this particular alcohol (reaction (2)) is not described in the literature. However, the rearrangement of similar compounds, e.g., 2-nitro-9-fluorenol to 7-nitrophenanthridine,[39] and 2-amino-3-nitro-9-fluorenone to 6-nitro-7-aminophenanthridine,[40,41] has been reported. In the former reaction it was reported that only a small amount of 2-nitrophenanthridine was obtained. Rearrangements with groups other than the nitro in the ring were less specific and mixtures of the 2- and 7-substituted phenanthridines were obtained. By analogy, we have assumed that the product we obtained by this rearrangement (reaction (2)) has the amino group in the 7-position.

The reaction was carried out in a suspension of NaN_3 and 98% H_2SO_4 in $CHCl_3$. The fluorenol was added as a fine powder. The work-up procedure was the same as described in the literature cited.[39] The elementary analysis of the purified product checked with the theoretical values calculated for 6-N,N-dimethylamino-7 nitro phenanthridine.

Details are given in the appendix.

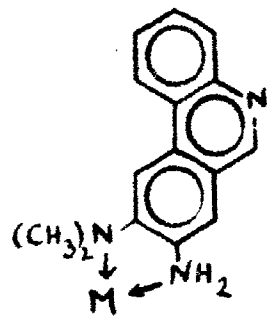
5.7 Attempt to Prepare N-[7-(6-Dimethylamino)-Phenanthridyl]-N' (Dimethyl) Urea. (MEL-264)



The starting material, 6-N,N-dimethylamino-7 nitro phenanthridine, was prepared by a series of reactions described under subsection 5.6.

A number of attempts were made to reduce the nitro group to the corresponding amine. The amounts used for the reaction were from 0.2 to 0.5g. First, we followed a preparation from the literature[39] where 7-nitro-phenanthridine was reduced to the corresponding amino compound by using Fe-powder in 0.05 normal acetic acid. Since the yield was very small, we tried several other methods, such as catalytic hydrogenation on Pt-char-

coal and Pd-charcoal. In these cases, we obtained only a small amount of material which would not crystallize. The small yield might be due to complex formation with the transition metals. From the structure one might expect quite a strong tendency toward complex formation.



Finally, we did some experiments using $Na_2S_2O_4$ as the reducing agent. One mole of nitro compound and 3 moles of $Na_2S_2O_4$ were refluxed in a mixture of H_2O-EtOH. After evaporation of the solvent, the residue was extracted with $CHCl_3$ to give an orange solid, which was reacted with dimethylcarbamyl-chloride without further purification. However, some inorganic residue was left after combustion; therefore, we could not yet obtain good analysis.

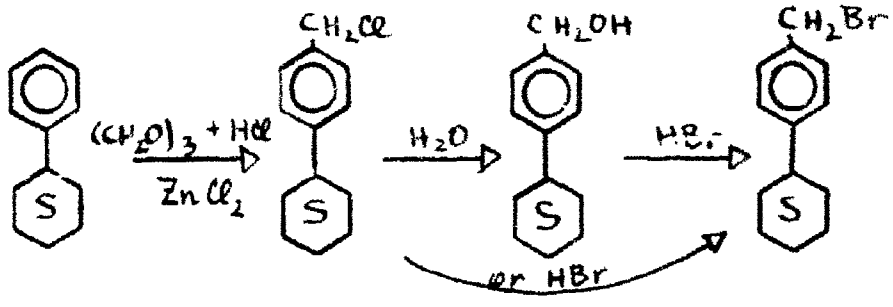
5.8 Attempted Preparation of MEL 340

Two attempts were made to prepare the compound. Equimolar amounts of glycolate and tropine were heated to 110°C with: (1) CH_3ONa and (2) Na (each in catalytic amount) for: (1) 2-1/2 hours and (2) 20 hours. To remove the methanol formed during the reaction, the system was maintained under vacuum (15 mm Hg). From the first reaction, a hydrobromide salt was prepared and isolated. However, analysis indicated it was simply tropine hydrobromide. From the second reaction we prepared and isolated a fumarate salt which again proved to be a salt of tropine.

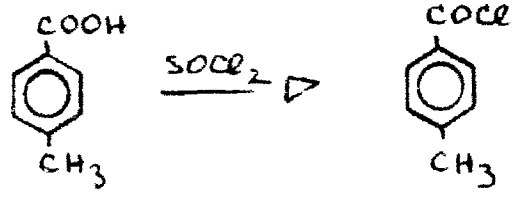
It appears that either the MEL 340 does not form the salts easily, so that only the tropine salts were isolated, or the transesterification did not take place under the reaction conditions investigated thus far. Another competitive reaction might explain why we are experiencing difficulty in obtaining the product in good yield, i.e., the OH group of the glycolate might undergo a transesterification with itself thus causing a self-polymerization.

5.9 p-Methyl-N(p-cyclohexylbenzyl) Tropyl Benzoate-Halide (MEL 341)

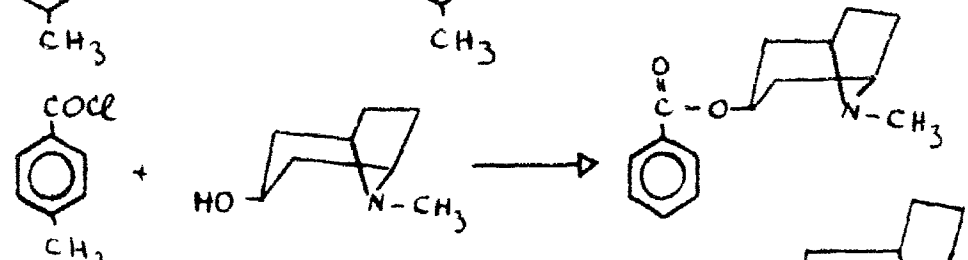
Proposed reactions:



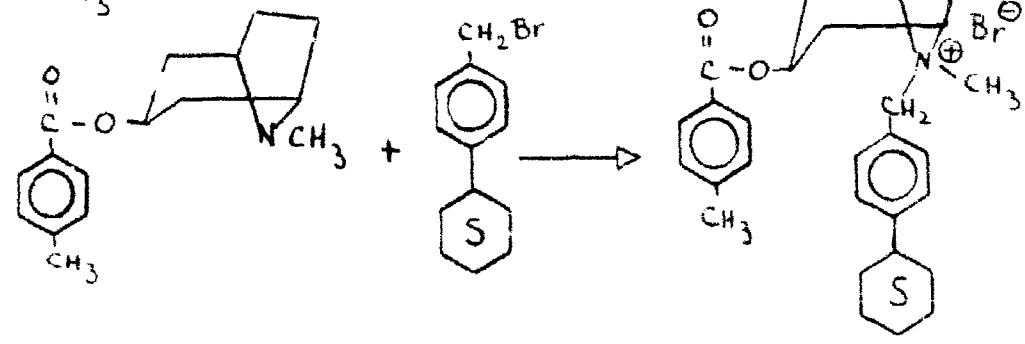
(1)



(2)



(3)

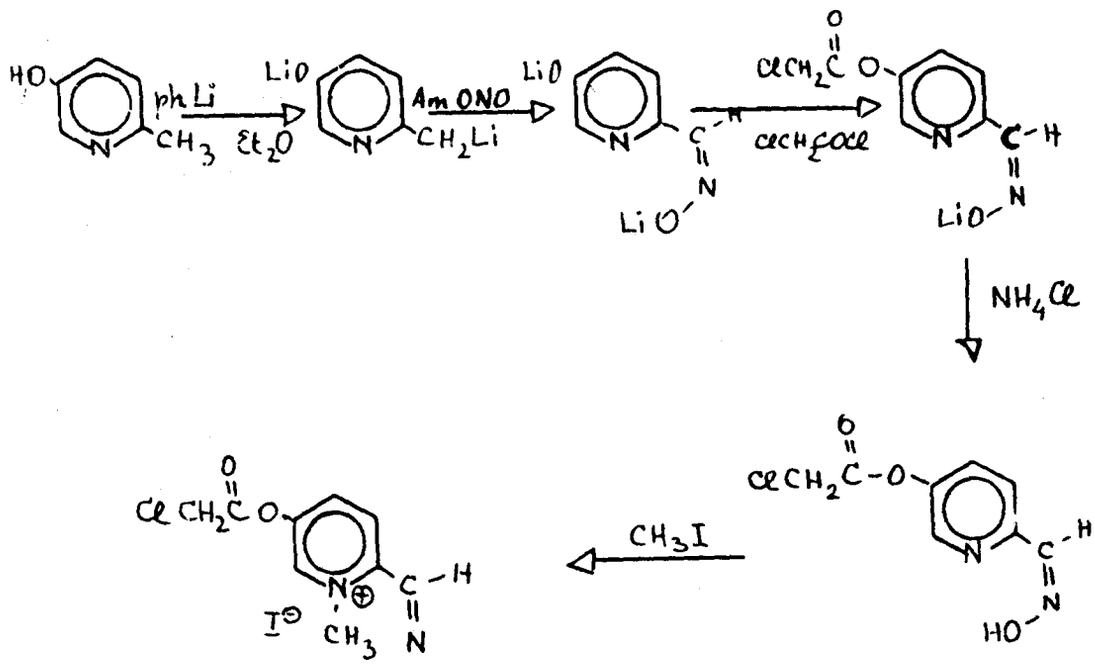


(4)

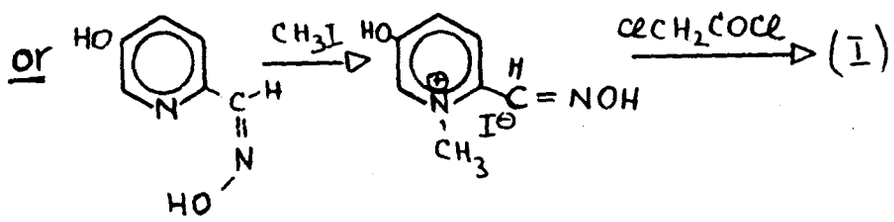
Direct bromomethylation of benzene, toluene, etc., has been described in the literature. We tried to extend this method to cyclohexylbenzene, but the final product decomposed during vacuum distillation. To isolate cyclohexylbenzylbromide, we therefore will try an alternate method which avoids distillation.. We will first prepare cyclohexylbenzychloride (by chloromethylation), and without further purification, hydrolyze with water to the corresponding benzylalcohol which can be distilled without decomposition. Refluxing with 40% HBr should give cyclohexybenzylbromide, which can then be extracted with ether. It is anticipated that the product will be pure enough to be used without further purification. Reaction of this compound with tropyl p-methylbenzoate should give the desired product.

p-toluoylchloride was prepared from p-toluic acid by refluxing with excess $SOCl_2$ in benzene and subsequent fractional distillation under reduced pressure. Tropine was then refluxed with a slight excess of p-toluoylchloride in toluene for 16 hours. The resulting hydrochloride was filtered and washed with ether; and it gave a C,H analysis that checked with the calculated value.

A route was proposed for the synthesis of 5 - ω-chloroacetoxy-pyridine-2-aldoxime methiodide (MEL-147):



(I)



[illegible] as a starting material. [illegible], it was desirable to have a relatively easy, high-yield method for preparing this starting compound.

Sulfonation of α-picoline and subsequent alkali fusion of the 2-methylpyridine-5-sulfonic acid seems to be the simplest way to prepare 5-hydroxy-2-methylpyridine. We prepared the sulfonic acid by heating α-picoline for 24 hours to 220° in fuming sulfuric acid. Excess fuming sulfuric acid was distilled under reduced pressure, and the tarry residue was extracted with H_2O. After concentration of the aqueous solution, the acid was precipitated with ethanol.

2-Methylpyridine-5-sulfonic acid was then mixed with excess NaOH and heated to 300°C for 2-1/2 hours. Neutralization and extraction with ether gave 2-methyl-5-hydroxy pyridine.

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APPENDIX

EXPERIMENTAL WORK

(In which the subsection or paragraph numbers correspond to the numbered discussions in sections 4 and 5 of the report.)

[illegible]*

[illegible]

Cyclobutanecarbonyl Chloride

A mixture of 400 ml of phosphorus trichloride and 200 g (2.0 mole) of cyclobutanecarboxylic acid was placed in a 1000-ml flask fitted with a reflux condenser and drying tube, and the mixture was refluxed for 3 hours. After removal of the excess phosphorus trichloride at atmospheric pressure, the residue was distilled through an 18-inch vacuum jacketed Vigreux column. There was obtained 189.7 g (80% of theoretical) of cyclobutanecarbonyl chloride, b.p. 70°C/50mm.

In another experiment, the volatile reaction products were distilled away from the reactive phosphorus oxychloride solids and the yield in this case was only 64.5%.

Cyclobutyl Phenyl Ketone

A solution of 189.7g (1.6 moles) of cyclobutanecarbonyl chloride in 500 ml of anhydrous benzene was added dropwise with stirring over a 2-hour period to a refluxing mixture of 266 g (2.0 moles) of anhydrous aluminum chloride in 2100 ml of benzene. After the addition was complete, refluxing was continued for an additional 2-hour period. The mixture was allowed to stand overnight, then poured into 3 gallons of crushed ice. The layers were separated, and the water layer was saturated with salt before triple extraction with benzene (a total of 3 liters). The combined benzene solutions were concentrated at atmospheric pressure, and the residue distilled through

*The numbering system corresponds to the discussion in the subsections of sections 4 and 5. The IR-spectra of all delivered compounds have been sent with the samples and will not be included here.

[illegible] at reduced pressure. There was collected 235 g (92.5%) [illegible]cyclobutyl phenyl ketone boiling at 110°/5mm, $n_D^{25°}$ = 1.5451.

Cyclobutyl-1-Phenyl -2-Propyne-1-ol

A 3-liter, 3-necked flask, fitted with a gas inlet tube, a dry-ice cooled condenser attached to a drying tube, and a mechanical stirrer, was charged with 2000 ml of anhydrous liquid ammonia and 42.2 g (1.83 gram atom) of sodium. When the sodium was dissolved, acetylene was passed in until the blue metallic color disappeared, then for an additional 10 minutes. The gas inlet tube was removed and replaced with a dropping funnel filled with 235 g (1.47 moles) of cyclobutyl phenyl ketone in 350 ml of anhydrous ether. This was added over a period of 2 hours; the mixture was stirred an additional 4 hours, and the ammonia was allowed to evaporate overnight. The ethereal residue was poured over 3 liters of crushed ice which was then acidified with dilute hydrochloric acid. The layers were separated, the aqueous phase extracted once with ether, and the organic phase was dried with anhydrous calcium sulfate, filtered, and concentrated. Distillation of the residue through a column gave 228 g (83.5%) 1-cyclobutyl -1- phenyl-2-propyn- 1-ol, b.p. 100°/2mm, $n_D^{25°}$=1.5460.

Cyclobutylphenylglycolic Acid

In one experiment, a solution of 335 g (2.12 moles) of potassium permanganate in 5.5 liters of warm water was added dropwise to a vigorously stirred mixture of 140 g (1.33 moles) of 1-cyclobutyl -1-phenyl-2-propyne-1-ol and 600 ml of water. Since great care was taken to keep the reaction mixture at 0° to 5° during the addition, a total of 8 hours was allowed for this step. After an additional 2 hours of stirring, the excess potassium

permanganate was removed with sodium thiosulfate. The mixture was filtered through a pad of Celite (a very slow process), the manganese dioxide was washed with an additional 1-liter portion of water made mildly basic with sodium hydroxide. After one washing with 500 ml of ether, the clear aqueous phase was acidified with dilute hydrochloric acid and saturated with salt. The white solid which appeared was separated by filtration, and the filtrate was extracted twice with 250-ml portions of ether. Evaporation of the ether gave a white solid, which was added to the residue in the filter funnel. There was obtained 65 g (28%) of cyclobutylphenyl glycolic acid as a white crystalline solid, m.p. 140°-141°C.

In another experiment, stirring at room temperature after addition of the potassium permanganate was increased to 15 hours. The yield of acid was raised slightly--to near 40%. When this modification was applied to a larger scale experiment, the expected 40% yield did not materialize--a 30% yield was the result. In still another experiment, an attempt was made to increase the homogeneity of the reaction mixture by dissolving the acetylenic compound in acetone before treatment with the aqueous permanganate solution. A yield of 30% of cyclobutylphenyl glycolic acid was the result of this modification.

In view of the apparent inefficiency of permanganate oxidation as a route to the glycolic acid, a short investigation of the anodic voltammetry of 1-cyclobutyl-1-phenyl-2-propyne-1-ol was carried out to determine the feasibility of an electrochemical oxidation to the glycolic acid. Studies of the anodic polarography of the acetylenic derivative in mixed water-methanol at various pH showed no wave for the compound. It is apparent that electrochemical oxidation of this material would require extensive study to be

useful on a preparative scale as a route to the substituted glycolic acid. It is, however, entirely reasonable to assume that an electrochemical process can be applied to this problem in view of the reported oxidation of acetylene to potassium formate in KOH medium at a platinum electrode.

Methyl Cyclobutylphenylgycolate

Cyclobutylphenyl glycolic acid, 38 g (0.184 moles) was dissolved in 100 ml of ether and added to an ethereal solution of 0.3 moles of diazomethane prepared from N-nitroso-N-methyl-p-toluensulfonamide ("Diazald", Aldrich Chemical Co., Inc.). The solution was allowed to stand overnight, the excess diazomethane destroyed with acetic acid, and the ethereal solution extracted with 10% solution of sodium bicarbonate. The residue left after the ethereal solution was dried with magnesium sulfate, filtered, and the ether evaporated off was recrystallized from benzene-petroleum ether to give 37.5 g (92.5%) of methyl cyclobutyl phenylglycolate, m.p. 51°-53°. Overall yield -- 17%.

4.2 Preparation of 1-(2-Oxypyrrolidino)-4-Pyrrolidion-Butyne-2 (Mel-150)

Preparation A: 1-Propargyl-2-Pyrrolidinone

2-pyrrolidinone, 30 grams (0.352 moles), was dissolved in 100 ml of dry toluene and added dropwise with stirring to a solution of 18 g (0.4 moles) of sodium hydride-mineral oil mixture in 200 ml dry toluene. The reaction mixture was refluxed for 2 hours and cooled. Propargyl bromide, 48 g (0.4 moles) in 100 ml of dry toluene was added with stirring over a period of 2 hours; the reaction mixture was heated for a further 2-hour period and allowed to stir overnight at room temperature. After filtration through Celite and evaporation of excess toluene, a dark oil was obtained. This was placed on an alumina column and eluted with a 1:1 heptane-ether mixture.

The resulting solution was concentrated under vacuum to give 1[illegible] g (57%) of a light yellow oil. This material darkens on standing, and when heated in air tends to polymerize to a black solid mass.

1-(2-Oxypyrrolidino)-4-Pyrrolidino-Butyne-2

A mixture of 9.6 g (0.08 moles) of the oil obtained above, 7.6 ml (0.088 moles) pyrrolidine and 2.88 g (0.096 moles) paraformaldehyde in 25 ml dioxane was heated at reflux for 16 hours. The dark reaction mixture was cooled and poured into 300 ml of water. After acidification with hydrochloric acid and thorough extraction with ether to remove unchanged pyrrolidine, the water solution was made basic again with sodium hydroxide. The organic material was extracted four times with chloroform (a total of 300 ml), and the chloroform solution was dried over $CaSO_4$, filtered, and concentrated under reduced pressure. There remained 14 ml of a dark-yellow oil which was placed in a modified Claisen apparatus and distilled at 1-mm pressure. Two fractions were collected: a 3-ml fraction at 165°-175° and a 2-ml fraction at 190°-225°. The main portion of the material remained behind in the form of a tarry material which solidified to a hard black mass on cooling.

Infrared analysis of both volatile fractions showed certain differences in structure, though both exhibited strong carbonyl absorption at 1685 cm^{-1}. A picrolonate salt, melting at 158°-160° after recrystallization from acetone, was prepared from the first fraction.

It appears that the low yield results from isomerization and polymerization processes arising in the distillation steps. Such behavior is common in acetylene derivatives and can be minimized by avoiding high temperatures. Chromatographic purification is probably applicable in this preparation.

Preparation B: To 64.5 g of pyrrolidine in 215 ml of toluene, a solution of 38.7 g of 55% NaH (mineral oil suspension) in 430 ml of toluene was added dropwise. The mixture was refluxed for 2 hours and then cooled. Propargyl bromide (103 g) was added over a period of 1 hour and refluxed for 1.5 hours. The filtered red-brown solution was evaporated under reduced pressure, placed on an Al_2O_3 column, and eluted with hexane and hexane-ether (1:1). The eluate was evaporated under reduced pressure to give a 2-phase oil; the lower, reddish liquid was separated (80.2g) from the top, colorless oil (16.4 g mineral oil). Upon refrigeration, 0.3 g of slightly yellow crystals precipitated (m.p., 185°-6°C). A mixture of 80.2 g of the red liquid, 63.5 g pyrrolidine, 24.0 g of paraformaldehyde, and 150 ml of dioxane was refluxed for 20 hours under an argon atmosphere. The liquid was then poured into 1200 ml of distilled H_2O, acidified with HCl to pH 3.5, and extracted 4 times each with 250 ml ether. The aqueous solution was brought to pH 10 and extracted with $HCCl_3$. The chloroform was evaporated under reduced pressure, the residual oil was degassed under oil pump vacuum, and then distilled with a molecular still. The wall temperature was 160°, and the pressure was 100 to 500 microns. The yield was 36.0 g of an orange oil. The crystals (m.p., 180°-5°C) were analyzed for C, H, N. Oxygen was calculated. The analyses fit an empirical formula C_7H_9NO.

Analysis: Calculated for, C_7H_9NO: C--68.4%, H--7.32%, N--11.39%; found: C--68.26, H--7.52, N--11.3%.

4.3 Preparation of Chloroacetylglycylglycine Ethyl Ester (Mel-131)

A mixture of glycyl glycine (10 g) and ethanol (250 ml) was placed in a 500-ml round-bottom flask equipped with stirrer, condenser, thermometer,

and gas addition tube. The mixture was stirred while gaseous anhydrous hydrogen chloride was passed into the reaction mixture. The gas addition was continued until the ethanol solution gave a positive acid test with pH indicating paper. The reaction mixture was then heated rapidly to reflux temperature and held there for 5 minutes. Practically all of the solids went into solution which was then filtered and cooled as rapidly as possible to -30°. White needles crystallized from the filtrate and were removed by filtration. This solid product was placed in a 250-ml beaker; 10 ml of water and 30 ml of chloroform were added and mixed to give a fine slurry. A sodium hydroxide solution containing 3.2 of sodium hydroxide in 8 ml of water was added with cooling and stirring. Potassium carbonate was added to saturate the water layer. The organic layer was separated and the water layer washed three times with 30-ml portions of chloroform. The chloroform solution was filtered and the filtrate concentrated to 15 ml, then poured into 250 ml of petroleum ether. White needles (m.p. 87°-88°) precipitated and were filtered off. This product was redissolved in 30 ml of chloroform(a) and added dropwise to a solution of chloro acetyl chloride (7.5 ml) in 30 ml of chloroform at 0°. After the addition a white solid began to precipitate. After standing for 1 hour, the mixture was poured into 500 ml of petroleum ether. The solid(b) formed was filtered off and recrystallized from acetone; 1.5 g of white needles (m.p. 151°-152°) were recovered.

Notes:

(a) The glycyl glycine ethyl ester decomposes on standing and must be used immediately.

(b) The solid was found to be a mixture of the desired product and glycyl glycine ethyl ester hydrochloride. The latter, which was completely insoluble in acetone, was removed by filtering the hot acetone solution.

References:

1. Ber. 34:2872 (1901)

2. Ber. 36:2113 (1903)

Characterization: $C_8H_{13}ClN_2O_4$; M.W. 236.67; m.p. 151-152°. (literature, m.p. 153°-154°).

4.4 Preparation of Chloroacetyl Glycyl Glycine Propyl Ester (Mel-132)

Glycyl glycine (0.1 m) was placed in a 500-ml round-bottom flask equipped with stirrer, thermometer, condenser, and gas addition tube. Propanol (250 ml) was added and the mixture was stirred while passing hydrogen chloride gas into it. The gas addition continued until the alcohol gave a strong acid indication with pH indicating paper. The mixture was then heated to reflux and held at that temperature until all went into solution. The solution was then filtered and the filtrate cooled to -30° for 2 hours. Twenty-six grams of white needles (m.p. 158°-159°) were recovered. The solid was dissolved in 30 ml of chloroform and 10 ml of water, and 8 ml of sodium hydroxide solution containing 0.4 g of sodium hydroxide per ml. Potassium carbonate was added to saturate the water, and the chloroform layer was separated. The water layer was washed three times with 30-ml portions of chloroform, and the organic layers were combined and concentrated to 15 ml. This was then poured into petroleum ether with stirring, and the resulting white solid filtered. It was immediately redissolved in chloroform (40 ml) and placed in a 100-ml, three-neck, round-bottom flask with stirrer and thermometer. Chloroacetyl chloride (3 ml) was dissolved in 15 ml of chloroform and added dropwise while maintaining the temperature at 0°. A precipitate formed, and after the reaction mixture had stood for 1 hour, it

was filtered. The solid* was recrystallized from acetone yielding 1.5 g of white powder (m.p. 127°-128°).

*Note:

The solid formed was found to be a mixture of two compounds. One was the desired product; the other was found to be glycyl glycine propyl ester hydrochloride, which was insoluble in hot acetone.

Characterization:

Analysis: Calculated for $C_9H_{16}ClN_2O_4$: C--43.0%, H--6.41%, Cl--14.15%, N--11.13%; found: C--43.86%, H--6.31%, N--11.01%, Cl--13.76%.

4.5 Preparation of Chloroacetyl Glycyl Glycine Methyl Ester (Mel-130)

Potassium hydroxide (5 g) was dissolved in 8 ml of water and placed in a 100-ml, three-neck, round-bottom flask equipped with thermometer and distillation head. Ethanol (25 ml) was added and the mixture heated to 65°. N-methyl-N-nitroso-p-toluenesulfonamide (10.8 g) was dissolved in 65 ml of ether and added dropwise, keeping the pot temperature 45° to 65°. The ether distillate and the diazomethane produced were collected in traps immersed in an ice bath. Ether was added to the reaction flask until the distillate became clear white. The yellow distillate was poured into a 250-ml Erlenmeyer flask containing choroacetyl glycyl glycine (5 g) which was stirred during the addition. The stirring was continued for 3 hours, then the ether solution was filtered to recover the solid product which was crystallized from acetone to obtain 3 g of white needles (m.p. 155°-156°).

Analysis: Calculated for $C_7H_{11}ClN_2O_4$: C--37.8%, H--4.98%, Cl--15.93%, N--12.59%; found: C--38.2%, H--5.18%, Cl--15.87%, N--12.31%.

Reference: "Preparation of Diazomethane from 'Diazald'", Aldrich Chemical Co., Inc., 2369 North 25th St., Milwaukee, Wisconsin.

4.6 Preparation of α-Chlorobenzaldoxime (Mel 210)

Benzaldoxime (50 g) was dissolved in chloroform (100 ml) and placed in a 25-ml, three-neck, round-bottom flask equipped with a magnetic stirrer, Drierite tube, and a gas addition tube.* Dry chlorine was passed into the ice-cold reaction mixture for 1 hour. The reaction mixture was allowed to come to room temperature, and the excess gases were allowed to escape upon the completion of the reaction through a Drierite tube protecting the flask. The solution containing the product was evaporated to dryness under reduced pressure. The crude product that remained in the flask was then recrystallized from petroleum ether to give α-chlorobenzaldoxime (9.6 g.; 14.9%) m.p. 48°-49° (lit., m.p. 48° or 52°).

*Notes:

(1) It is very necessary to keep the reaction mixture between 5°-10°C during the addition of dry chlorine.

(2) The reaction mixture should be protected by a Drierite tube during the reaction.

Characterization: The melting point corresponded to that given in literature; therefore, no elementary analysis is given.

Reference: B. Werner, Ber. 27(2197) (Beilstein Organische Chemie, 9, 316(129)).

4.7 Preparation of 1,3-Bis (triethylammonium) Propane Dibromide (Mel-171)

A solution of 10.1 g (0.05 mole) of 1,3-dibromopropane (Calbiochem), 25 ml of redistilled triethylamine and 10 ml of anhydrous methanol was refluxed in the dark for 72 hours. The reflux condenser was topped with a drying tube filled with Ascarite to exclude moisture and CO_2.

The crystalline material was filtered off, washed with ether, and recrystallized twice from alcohol and ether mixtures to give 6.1 g (31%) of 1,3-bis (triethylammonium) propane dibromide, a white crystalline material, m.p. 222°-24°, soluble in water, ethanol, and acetone.

Calculated for C_{15} H_{36} Br_2N_2: Br--39.6%; found: Br--39.3%, and 39.8%.

Reference: Barlow and Ing. Nature, 161, 718 (1948).

4.8 Preparation of L-1-Tosylamido-2-Phenylethyl Chloromethyl Ketone (TPCK) (Mel-180)

1. Preparation of N-Tosyl-L-Phenylalanine: Freshly recrystallized p-toluenesulfonyl chloride, 14.2 (0.072 moles) was dissolved in 75 ml of ether and shaken for 4 hours at 5° with 10 gram (0.06 mole) of L-phenylalanine (Calbiochem) in 25 ml of 2 N sodium hydroxide. The ethereal and water layers were separated, the aqueous phase acidified with 50% hydrochloric acid, and the crystalline product isolated by filtration. Recrystallization from 75% ethanol gave 18.2 grams (85%) of N-tosyl-L-phenylalanine, m.p. 158°-160°.

2. Preparation of N-Tosyl-L-Phenylalanyl Chloride: A solution of 16.2 (0.05 moles) N-tosyl-L-phenylalanine and 12.5 g of phosphorus pentachloride (from a freshly opened bottle) in 200 ml of anhydrous ether was stirred at 0° for 30 minutes, at room temperature for 1 hour, and finally allowed to stand at 0° overnight. Concentration of the ethereal solution gave a crystalline product, which was separated by filtration and washed with a little ether, then with ice water. After drying for 4 hours in a vacuum desiccator, 7.85 (46%) of the acid chloride was obtained. It melted with decomposition at 129°.

(0.023 moles) was suspended in 100 ml of anhydrous ether and treated with 0.07 moles of diazomethane in 300-ml anhydrous ether. The diazomethane was prepared from N-methyl-N-nitroso-p-toluenesulfonamide (Aldrich Chemical Co. "Diazald"). After standing overnight, the reaction mixture was refluxed for 30 minutes. In one experiment the diazomethyl ketone was isolated and showed the characteristic peak at 4.68 μ in the infrared. The ethereal solution of the diazomethyl ketone was treated with hydrogen chloride by bubbling in the dry gas for 3 hours. After evaporation of the ether, the residue was recrystallized, once from 1-1 benzene-hexane mixture and twice from 95% ethanol, to give 4.5 g (56% yield) of N-tosyl-amido-2-phenylethyl chloromethyl ketone, a white crystalline solid, m.p. 102.5°-103°.

References:

1. E. Fischer and W. Lipschitz, Ber. 48, 360 (1915)
2. F. A. Popenoe and V. D. deVigneand, J. Am. Chem. Soc. 76, 6202 (1954).
3. G. Schoellmann and E. Shaw, Biochem. 2, 253 (1963)

4.9 Preparation of 4-Chlorobutyroyl Chloride (Mel-282): This compound was commercially available from Aldrich Chemical Company (100 g).

Physical Constants: $B.P._{12}$ 66-67° (lit., $B.P._{12}$ 60-61°)

n_D^{20} = 1.4606 (lit., n_D^{20} = 1.4616

Reference: Blicke, Wright, Zienty, Am. Soc. 63 (1941) 2489 (Beilstein Organische Chemie E III 2, 627).

4.10 Preparation of 3-Chlorobutyryl Chloride (Mel-283)

Freshly distilled thionyl chloride (37.5 g) was placed in a 250-ml, three-necked, round-bottom flask equipped with a magnetic stirrer, dropping funnel, thermometer, and water condenser. The HCl given off during the

reaction was absorbed in a water trap attached to the condenser through a gas outlet tube. While stirring and gently warming the thionyl chloride, 3-chlorobutyric acid (22 g) was added dropwise during the course of 30-40 minutes. The HCl was collected in the trap as described above, and the sulfur dioxide was permitted to escape into the hood. When all of the acid had been introduced, the reactants were heated for an additional 1/2 hour on a water bath. The crude oily product remaining in the flask was purified through distillation at reduced pressure to yield pure 3-chlorobutyryl chloride (10.3 g; 36.7%); BP_{12} 47-48° (lit., BP_{12} 40°-41°); n_D^{20} = 1.4500 (lit., $n_D^{20.05}$ = 1.4509).

Characterization: The boiling point and refractive corresponded to that given in the literature.

References:

1. B. Michael, Ber 34 4051 (Beilstein Organische Chemie 2, 278).

2. Abderhalden, Fleischmann, Fermentf. 10, 203 (Beilstein Organische Chemie E II 2, 253).

3. H. Gilman and S. A. Harris, "Organic Synthesis," Coll. Vol. I, 2nd Ed. (1947) 147.

4. A. I. Vogel, A Textbook of Practical Organic Chemistry Including Qualitative Organic Analysis, 3rd Ed. (1956) 386-89.

4.11 Preparation of 4-Bromobutyryl Bromide (Mel-281)

Butyrolactone (30 g) and phosphorus tribromide (73 g) were placed in a 100-ml pear-shaped flask and heated on the steam bath for 2 hours under anhydrous conditions. The reaction was completed when the temperature was

brought up to 180° in an oil bath. The crude, oily product was distilled under diminished pressure, yielding the pure 4-bromobutyryl bromide (35.5 g, 57.1%); *B.P.$_5$ 70°-72°(lit., B.P.$_{13}$ 90°-91°); $n_D^{17.5}$ = 1.5346 (lit., not given).

Characterization:

Elemental Analysis: $C_4 H_6 Br_2$: Calculated: C--20.9%, H--2.62%, Br--69.5%; found: C--20.3%, H--2.70%, Br--66.8%.**

Notes:

*Compound was distilled at 5-mm Hg instead of 13-mm Hg since some polymerization of unreacted butyrolactone appeared to occur at the higher temperature required for distillation at 13-mm Hg.

**Analytical difficulties in obtaining good results in the bromine analysis were due to the rapid hydrolysis of the acid halide.

Reference: Kuschinsky, G. Lange, Scholtissek, and Turba, Biochem. Z. 327, 314-30 (1955).

4.12 Preparation of Isonitrosoacetone (Mel-231)

Ethyl acetoacetate (200 g), KOH (100 g), and H_2O (3750 ml) were placed in a 4-liter beaker; the mixture was allowed to stand for 18 hours. To this mixture sodium nitrite (124 g) in H_2O (400 ml) was added, and the solution was chilled to 5°-6°C in an ice-salt bath. Twenty percent (by weight) H_2SO_4 (860 g) was added dropwise during the course of 1 hour. After 15 minutes, the solution was neutralized with 30% NaOH. While keeping the solution cold 5°-6°C) the sodium salt of isonitrosoacetoacetate was acidified with 20% H_2SO_4 to pH 4. The solution containing the final product was allowed to warm to room temperature. The product was then extracted with ether and

dried over anhydrous sodium sulfate. The etheral extract was filtered from the Na_2SO_4 and evaporated to dryness. *The crude product (105 g, 80%) was recrystallized from benzene yielding crystalline plates of isonitrosoacetone (70.52 g, 54.2%) m.p. 66°-67° (lit., m.p. 45° or 69°).

*Note:

It is necessary to store the final product in a dark bottle and to keep cold.

Characterization: The melting point corresponded to that given in the literature.

Reference: V. Meyer, Zublin, Ber. 11, 695; Ceresole, Ber. 15, 1327; Charrier, Gazz. 37 II, 145 (Beilstein 1, 763).

4.13 Preparation of α-Chloro-α-Isonitrosoacetone (Mel-230)

A calculated amount of chlorine (13.4 g plus a 3.8 g excess) was dissolved in chloroform (150 ml).* A solution of isonitrosoacetone (15 g) in chloroform (50 ml) was placed in a three-neck, round-bottom flask equipped with a magnetic stirrer, thermometer, and dropping funnel. The chlorine solution was placed in the dropping funnel and added dropwise to the isonitroso acetone mixture.* After introducing all of the chlorine, the reactants were allowed to stir for 1 hour. A white precipitate was formed, which dissolved in the chloroform when allowed to warm to room temperature. The chloroform solution containing the final product was evaporated to dryness, and the crude product (21 g) was recrystallized twice from benzene

yielding a slightly yellow powder, α-chloro-α-isonitrosoacetone (3.7 g, 17.6%) m.p. 108-109° (lit., m.p. 107° or 109°).

*Note:

It is necessary to keep the temperature between 0°-10°C during the addition of the chlorine to the chloroform. The chloroform solution of chlorine should be added at such a rate that the temperature of the reaction mixture does not exceed 10°C.

Characterization: The melting point corresponded to that given in the literature.

Reference: Claisen, Manasse, Ann. 274, 98; Ponzio, Charrier, Gazz. 37 II, 68; (Beilstein III, 620).

4.14 Preparation of 2-(α-Chloro) Pyridinealdoxime Hydrochloride (Mel-220)

Twelve and 2 tenths grams of 2-pyridinealdoxime were dissolved in 170 ml of $CHCl_3$ (dried with $MgSO_4$). In a three-neck flask with stirrer, thermometer, and dropping funnel, the solution was cooled down to -55°C, then a solution of 8.4 g of chlorine in 100 ml of dry $CHCl_3$ was added dropwise while the reaction mixture was stirred vigorously. After addition of the chlorine solution, the reaction mixture was allowed to warm up to room temperature. The white powder which precipitated during the reaction was filtered quickly and dried under vacuum. Yield--18.5 g (96%); m.p. (sealed tube) 173-7° decomp.

Characterization:

Elemental Analysis: $C_6 H_6 Cl_2 N_2 O$: Calculated: C--37.3%, H--3.11%, Cl--36.8%, N--14.5%; found: C--37.7%, H--3.47%, Cl--36.9%, N--14.4%.

4.15 Preparation of 4-Trimethylammonium Methylbutyrate Bromide (Mel-174)

4-Bromobutyric Acid: Twenty four and six tenths grams of 4-bromobutyronitrile were refluxed for 2-1/2 hours with 67 g of 48% HBr. Excess HBr was then evaporated off under vacuum. The precipitated NH_4Br was filtered and washed with CCl_4. The filtrate was extracted five times with 50-ml CCl_4 each. The combined CCl_4 extracts were dried and evaporated under vacuum. The product crystallized upon cooling, yielding 22.5 g of 4-bromobutyric acid, m.p. 32°C.

4-Bromomethylbutyric acid was treated with a solution of CH_2N_2 in ether until the yellow color remained for 15 minutes. Excess CH_2N_2 was destroyed with a few drops of glacial acetic acid until color was only slightly yellow. The ether was evaporated under reduced pressure to give a water white residual liquid (34 ml).

4-Trimethylammonium Methybutyrate Bromide: Without further purification the ester was placed into a 250-ml autoclave. A solution of 30 ml of MeOH and 15 g of trimethylamine (the mixture was prepared with cooling) was added. The mixture was heated at 70°C for 24 hours. MeOH was evaporated under reduced pressure; the white solid which crystallized was recovered with 30 g overall yield, 75% based on butyronitrile. The compound was further purified by dissolving it in a minimum amount of MeOH and precipitating with ether-acetone (6:1). The white crystals were washed with acetone and dried. The compound did not have a real melting point, it softened at 120 -5° and decomposed without giving a clear melt up to 230° because of the possible formation of $(CH_3)_3N$-HBr or NH_4Br. The compound dissolves in H_2O, MeOH; it is insoluble in ether and is slightly soluble in acetone.

Characterization:

Elementary Analysis: $C_8 H_{18} Br O_2 N$: Calculated: C--40.0%, H--7.5%, Br--33.3%; found: C--40.0%, H--8.5%, Br--33.1%.

Discussion: The procedure in the literature[1] was not very complete and no yield was given for the ester. In order to avoid splitting off HBr during esterification with H_2SO_4 as a catalyst, we used CH_2N_2. Without further purification, the ester was used for the quaternization. As a solvent for the quaternization reaction, MeOH was used instead of EtOH to prevent possible transesterification. The melting point of the quaternary salt in the literature[1] was given as 82°, which is not in agreement with the melting point found by us (120°-5° decomp.). This fact might be explained as follows.

The authors of the literature cited[1] used ethanol as a solvent for the quaternization reaction. It is therefore easily possible that a base-catalyzed transesterification occurred to give the ethyl instead of the methylester. Or it is a mixture of both which would probably be hard to separate. A low melting point would be explained on this basis. The melting point, 82° (in the literature), seems rather low for a quaternary salt.

The authors[1] have for characterization of the compound a bromine analysis which corresponded more closely to the ethylester than to the methylester. They do not give a carbon and hydrogen analysis which would show the difference.

We assume that either the melting point in the literature is not correct or they obtained, not the methyl, but the ethylester.

Reference: (1) G. Aksnes and J. E. Prue, J. Chem. Soc. 103, 1959.

4.16 Preparation of p-Aminobenzyl Cellulose (Mel-320)

Preparation of p-Nitrobenzyl Cellulose: In a 2-liter flask equipped with mechanical stirrer, condenser, and thermometer, was placed 500 g of powdered cellulose (obtained from "SOLKA-FLOK" by washing with dilute acid, dilute alkali, water, and then drying)* and 600 ml of 20% NaOH.

One hundred grams of p-nitrobenzyl chloride was added, and the mixture stirred vigorously for 1-1/2 hours while heating to 95°C.

After cooling, the mixture was diluted with 3 liters of distilled H_2O and then neutralized with concentrated HCl. The mixture was filtered in a basket centrifuge, washed with H_2O, EtOH, and acetone. The red-orange solid was treated overnight with acetone in a Soxhlet extraction apparatus. Yield--54 g of p-nitrobenzyl cellulose.

Fifty four grams of p-nitro benzyl cellulose was suspended in 800 ml of distilled H_2O and heated to 80°C. One hundred twelve grams of $Na_2S_2O_4$ in 500 ml of H_2O were added dropwise while stirring. After addition of $Na_2S_2O_4$, the reaction mixture was heated for an additional 2 hours and then filtered. The solid obtained was washed twice with distilled H_2O, once with EtOH, and then dried. Yield--50.0 g p-aminobenzyl cellulose.

Characterization: Titration with a 1% $NaNO_2$ solution until the blue color of starch iodide remains for 1/2 hour: 0.5 milliequivalent/g.

4.17 Preparation of 5-Bromovaleryl Bromide (Mel-284)

5-Bromovaleric acid: 5-Bromovaleronitrile (14.5 g) and 40 g of 48% HBr were heated under reflux with stirring for 2-1/2 hours. After cooling the reaction mixture, the precipitated NH_4Br was filtered and washed thoroughly

*Proc. Nat. Acad. Sci., 37, 575 (1951).

with CCl_4. The aqueous layer was extracted several times with CCl_4, and all of the organic extracts and washings were combined. Most of the CCl_4 was evaporated under reduced pressure, and the crystalline 5-bromovaleric acid was filtered off and dried. Yield--12.5 g (77% of theory) m.p. 32-34°C.

5-Bromovaleryl Chloride: 5- Bromovaleric acid (12.5 g) was heated at reflux with 10 g of $SOCl_2$ for hours. The reaction mixture was distilled at reduced pressure to obtain 5-bromovaleryl chloride. Yield--12.4 g (89.8% of theory).

5-Bromovaleryl Bromide: 5-Bromovaleryl chloride (12.4 g) was placed in a reactor with a gas inlet tube and kept cold while dry HBr was bubbled slowly into the reactor for a period of 3 hours. The reaction mixture was distilled at 1-mm pressure and 10 g of a fraction boiling at 75°-76°C was collected. Yield--66.1% of theory.

Characterization:

Refractive Index: $n_D^{24^\circ} = 1.5200$

Elementary Analysis*: $C_5 H_9 O Br_2$: Calculated: C--24.6%, H--3.28% Br--65.6%; found: C--25.7%, H--4.9%, Br--62.0%.

*Note: Because of the extreme sensitivity of the compound to moisture in the air, it was difficult to obtain a very accurate analysis.

References:

1. Chem. Abstrs., 45, 4644c (1951)

2. Standinger and Anthes. Ber., 46 II, 1417 (1913).

4.18 Preparation of 3-Bromopropionyl Bromide (Mel-285)

3-Bromopropionyl chloride (20 g) was placed in a reactor equipped with a gas inlet tube. Dry HBr was bubbled into the cooled reaction mixture for 3-1/2 hours. The reaction mixture was distilled at 20-mm pressure, and a fraction boiling at 70°-84°C was collected. Yield--9.4 g (37.2% of theory).

Characterization:

Refractive Index: $n_D^{20^\circ} = 1.5281$

Elemental Analysis*: $C_3 H_4 O Br_2$: Calculated: C-16.7%, H--1.86%, Br 74.3%; found: C--17.1%, H--2.2%, Br--72.7%.

*Note:

Because of the extreme sensitivity of the compound to moisture in the air, it was difficult to obtain a very accurate analysis.

Reference: Staudinger and Anthes, Ber., 46 II, 1417, (1913).

4.19 Preparation of 3-Bromobutyryl Bromide (Mel-280)

3-Bromobutyryl Chloride: 3-Bromobutyric acid (20 g) was heated at reflux with $SOCl_2$ (17.2 g) for 2 hours. The reaction mixture was distilled at 17-mm pressure, and the fraction distilling at 72°-82°C was collected. Yield--13.8 g (62.0% of theory).

3-Bromobutyryl Bromide: Dry HBr was bubbled through 13.8 g of cooled 3-bromobutyryl chloride for a period of 3 hours. The reaction mixture was distilled at 3 mm, and 12.1 g of a fraction distilling at 50°-60°C was collected. Yield--71.6% of theory.

Characterization:

Refractive Index: $n_D^{20^\circ} = 1.5089$

Elementary Analysis*: $C_4 H_6 O Br_2$: Calculated: C--20.9%, H--2.62%, Br--69.7%; found: C--22.3%, H--3.0%, Br--67.4%.

*Note:

Because of the extreme sensitivity of the compound to moisture in the air, it was difficult to obtain a very accurate analysis.

Reference: Staudinger and Anthes, Ber. 46 II, 1417 (1913).

4.20 Preparation of 4-Trimethylammonium Methyl Crotonate Bromide (Mel-172)

4-Bromo-Methylcrotonate: To a 500-ml, round-bottom flask equipped with stirrer and reflux condenser, was added a carbon tetrachloride solution of methyl crotonate (40 g) and N-bromosuccinimide (36 g), which had been purified by recrystallization from benzene. The reaction mixture was refluxed for 12 hours then cooled and filtered. The CCl_4 was removed at reduced pressure and the residual oil was distilled at 8 mm Hg. The fraction boiling at 77°-78° was 4-bromo methyl crotonate. (29 g, 81% of theory).

4-Trimethylammonium Methyl Crotonate: 4-Bromo methyl crotonate (11 g) in 15 ml of methanol and $(CH_3)_3N$ in 25 ml of methanol were rocked in autoclave at room temperature for 2 days. The solvent was evaporated, and the residual oil was diluted with 20 ml of acetone and scratched. Crystals formed and 100 ml of ether was added; the brownish crystals were filtered off and washed with 120 ml of hot acetone. The compound, after drying, weighed 12.7 g (87% of theory) and melted from 151° to 156°. The compound was purified by twice dissolving in a very small amount of methanol and reprecipitating with acetone-ether. (m.p. 153-6°).

Elemental Analysis: $C_8 H_{16} NO_2 Br$: Calculated: C--40.2%, H--6.7%; found: C--39.7%, H--7.4%.

Reference: Vogel, A Textbook of Practical Organic Chemistry, Longman Green and Co., London, 1948, p. 1004-5.

4.21 Preparation of α-Pyridinium Acetophenoneoxime Chloride (Mel 250)

α-Chloroacetophenoxime: A solution of α-chloroacetophenone (6.16 g = 0.04 moles) in 95% ethanol was combined with an aqueous solution of hydroxylamine hydrochloride (5.52 g = 0.08 moles). More ethanol was added, and the solution was heated slightly to dissolve all of the ketone. Upon cooling and the addition of more water, the oxime precipitated.

This product was recrystallized twice from carbon disulfide resulting in white crystals m.p. 83°-5° (Lit. m.p., 88.5°-89°C). Yield--2.69 g (40% of theory).

Characterization:

Elemental Analysis: $C_8 H_8 NOCl$: Calculated: C--56.68%, H--4.76%, N--8.26%, Cl--20.91%; found: C--56.35%, H--5.02%, N--7.22%, Cl--20.20%.

α-Pyridinium Acetophenoneoxime Chloride: α-Chloracetophenoxime (2.5 g = 0.15 moles) was dissolved in 200 ml toluene. Pyridine (3.16 g = 0.04 moles) was added, and a white solid formed immediately at room temperature. After refluxing the mixture with starting for approximately 3 hours, more white solid formed. This was filtered off and purified by dissolving in methanol and precipitating with anhydrous ethyl ether. The white crystalline solid thus obtained melted with decomposition starting at 182°C. Yield--2.40 g (65% of theory).

Characterization:

Elemental Analysis: $C_{13} H_{13} N_2 OCl$: Calculated: C--62.8%, H--5.22%, N--11.26%, Cl--14.26%; found: C--63.05%, H--5.58%, N--10.70%, Cl--12.75%.

4.22 Preparation of 4-Dimethyl Sulfonium Methyl Butyrate Halide (Mel-175)

4-Bromo-Butyric Acid: 4-Butyrolactone (86 g) and 420 ml of 48% HBr were refluxed for 2 hours. The HBr solution (130 ml) was distilled off under reduced pressure, and the residual liquid was extracted with CCl_4 (once with 100 ml and 3 times with 50 ml), dried, and decolorized with a small amount of charcoal. The filtered solution was evaporated to give 90 g of 4-bromobutyric acid (m.p., 32°C).

4-Bromo-Methybutyrate: The above acid (45 g) was treated with a solution of CH_2N_2 until the yellow color remained. After standing overnight, the solvent was evaporated to give 48 g of ester.

Unsuccessful Attempt: Part of the above ester (24 g) and 17 g of $(CH_3)_2S$ in 400 ml of MeOH were placed in a 250-ml autoclave and heated at 70°C for 64 hours. After cooling to room temperature, 800 ml of ether were added. White crystals precipitated, which were redissolved in MeOH and precipitated with ether. The yield was 19 g. The substance was recrystallized once more in the same way. There was no real melting point, but the substance changed color between 140° and 160°C and seemed to disappear between 170° and 180°C while droplets condensed on the wall of the sealed melting point tube. The substance was very soluble in water and MeOH, insoluble in ether, and only slightly soluble in acetone.

The elementary analysis corresponded, not to the calculated values for the desired compound, but very closely to those for trimethyl sulfonium bromide.

	Calculated for: $[(CH_3)_2S\text{-}CH_2CH_2COOCH_3]^{\oplus}Br^{\ominus}$	Calculated for: $[(CH_3)_3S]^{\oplus}Br^{\ominus}$	Found
C	34.6%	23.0%	23.73%
H	6.18	5.74	5.78
Br	32.94	50.0	49.1

Successful Preparation:

Sodium methylmercaptide: Sodium (11.5 g) was reacted with continuous stirring at -60° in 250 ml of methanol. A solution of 30 ml of CH_3SH in 30 ml of methanol, cooled to -60°, was added to the sodium methoxide solution

and the mixture was allowed to come to room temperature. Methanol was removed by vacuum evaporation to a volume of 120 ml. Toluene was added and the removal of solvent was continued under vacuum until no more methanol remained. The $NaSCH_3$ which precipitated from the reaction mixture was filtered and dried. Analysis of the sodium methylmercaptide by treatment with an excess of standard I_2 and by back titration with sodium thiosulfate indicated a purity of 100.5%.

4-(Methyl Butyrate) Methyl Sulfide: Methyl-4-bromobutyrate (10 g) and CH_3SNa (4 g) were dissolved in 100 ml of methanol and refluxed for 7 hours. After evaporation of the solvent in a rotary evaporator (bath temperature 35°), the residue was taken up in water and extracted with ether. The ether extract was dried, then distilled to remove solvent, and finally vacuum distilled to recover the thioether (B.P. 91°-92°/12mm).

4-Dimethyl Sulfonium Methyl Butyrate Iodide: 4(methyl butyrate) methyl sulfide (6.3 g) and methyl iodide (11.5 g) were mixed with 35 ml of acetone and allowed to stand for 2-1/2 days. Two hundred ml of dry ether were added and the white crystals which precipitated were filtered and dried. Yield--10.9 g (89% of theory) m.p. 64°-66°.

Characterization:

Elementary Analysis: $C_7 H_{15} SO_2 I$: Calculated: C--28.99%, H--5.28%; found; C--28.89, H--5.49%.

References:

1. M. Protiva and M. Borovicka, Czech. Pat. 90, 407, C.A. 54, 24935 h.

2. Ber. 83, 265 (1950).

4.23 Preparation of N-Benzoyl-l-Tyrosine-p-Nitroanilide (Mel-290)

p-Nitroaniline-hydrochloride was prepared by dissolving nitroaniline in benzene and bubbling in dry HCl gas. The precipitate was filtered, washed with benzene and anhydrous ether, then dried.

One gram of N-benzoyl-l-tyrosine amide and 0.62 g of p-nitroaniline hydrochloride were mixed and heated in a small flask, triturated with H_2O, decanted, and the remaining solid dissolved in EtOH. Water was added again and the yellow-brown product was filtered and washed with 1-1/2 liters of distilled H_2O. The remaining material was dissolved in EtOH, precipitated with H_2O, dissolved in ether, precipitated with hexane, and dried. Yield--0.5 g, m.p. 225 -28° uncorrected. Literature m.p. 226 -228°C.

Reference: Anal. Biochem, 3, No. 5, 431 (1962).

4.24 Preparation of 4-Dimethyl Sulfonium Methyl Crotonate Iodide (Mel-173)

4-(Methyl Corotonate) Methyl Sulfide: Methyl 4-bromo crotonate (15.1 g) and sodium methylmercaptide (6.5 g) were refluxed in 100 ml of methanol for 7 hours. The methanol was removed in a rotary evaporator (bath temperature, 35°) and the residue was taken up with water. The mixture was extracted with ether, the ether extract was dried, and the solvent evaporated. The residue was distilled under vacuum and the fraction distilling over at 77°-107°/11 mm was collected.

4-Dimethyl Sulfonium Methyl Crotonate Iodide (Mel-173): 4-(methyl crotonate methyl sulfide (3.9 g) and methyl iodide (7 g) in 15 ml of acetone were mixed and allowed to stand at room temperature for 2-1/2 days. A trace (0.26 g) of yellow crystals precipitated out m.p. 214°-215°. This compound is probably trimethyl sulfonium iodide. The solvent was removed

by evaporation under vacuum and the oily residue was treated with ether. No crystallization occurred even on cooling to dry-ice temperature. Water was added, and the aqueous layer was separated from the ether portion. The oil was very soluble in water and was soluble also in EtOH, MeOH, acetone, $CHCl_3$; it was insoluble in ether, hexane, CCl_4 and benzene. The water solution was evaporated in a rotary evaporator (bath temperature, 35°), and the residual oil was dried further in a vacuum desiccator over P_2O_5. The oil weighing 3.5 g failed to crystellize, but was submitted for elemental analysis.

Characterization:

Elemental Analysis: $C_7 H_{13} SO_2 I$: Calculated: C--29.2%, H--4.5%; found: C--29.6%, H--5.0%.

4.25 Preparation of 2-Fluorobenzaldoxime (Mel-222)

2-Fluorobenzaldehyde (13.9 g) and hydroxylamine hydrochloride (6.9 g) were mixed with 170 ml of water and 25 ml of ethanol. Then, Na_2CO_3 (7.3 g) was added, and the reaction mixture was stirred at room temperature for 7 hours. The organic layer was separated, and upon addition of water and scratching the oxime crystallized and was filtered off. After drying under vacuum, 12.0 g (86.3% of theory) was recovered. The compound was recrystallized from hexane to obtain white crystals, (m.p. 60°-62°; lit., m.p. 62.6°).

Characterization: The melting point corresponded to that reported in the literature.

Reference: Beilstein VII, First Supplement, p. 132.

4.26 Preparation of Pyrene Sulfonyl Chloride (Mel-121)

Pyrene sulfonic acid (0.005 mole, 1.47 g) and phosphorus pentachloride (0.005 mole, 1.14 g) were mixed and placed in a 100-cc, round-bottom flask equipped with condenser and drying tube.

The mixture was heated to 155°C, using an oil bath, and maintained at 155°C for 15 hours. The reaction mixture was then cooled under N_2 and added to 100 cc of ice-water mixture. The mixture was then filtered, and a yellow-solid was isolated, which was immediately dried under vacuum.

The dried yellow solid was dissolved in chloroform and precipitated by addition of n-hexane and scratching; no sharp m.p.--decomposes above 120°.

Characterization:

Elemental Analysis: $C_{16}H_9SO_2Cl$: Calculated: Cl--12.1%; found: Cl--11.8%.

4.27 Preparation of α Chloro-2-pyridinealdoxime methchloride (Mel-221)

2-pyridine Aldoxime Methchloride: Methanol (150 ml) containing 8.1 grams of dry HCl was added dropwise to 7.95 g of 2-pyridine aldoxime methiodide and 150 ml of methanol in a 500-ml, round-bottom flask. When the addition was completed, the mixture was concentrated by distillation to a volume of 30 ml. Upon cooling, a solid began to precipitate. Precipitation was completed by the addition of a 50-50 mixture of Et_2O and acetone. The solid was filtered and dried--m.p. 233° to 235°C (open block).

α Chloro-2-Pyridnealdoxime Methchloride (Sample A): 2-Pyridinealdoxime methchloride (2.3 g) and 100 ml of MeOH were placed in a 250-ml flask equipped with a mechanical stirrer, two addition funnels, and an ice-water bath. To this mixture (cooled to 0°C) was added simultaneously while stirring, a solution of sodium methoxide (prepared by reacting 0.31 g Na metal with 30 ml of MeOH) and 30 ml of MeOH saturated with Cl_2. After the additions were completed, the reaction mixture was allowed to warm up to ambient temperature and maintained at this temperature for 3 hours. Additional Cl_2 was then

passed through the mixture until a pronounced yellow color was obtained, and the reaction mixture was allowed to sit over night at room temperature. The reaction mixture was then filtered from NaCl, and the filtrate evaporated to dryness. The solid obtained was redissolved in a minimum amount of hot MeOH, filtered, and then cooled. Very fine crystals began to precipitate. Precipitation was completed by the addition of Et_2O. A second recrystallization from EtOH did not lower the melting point. Decomposed at 173°-180°C (uncorrected).

Characterization:

Elemental Analysis (Sample A): Calculated: C--40.75%, H--3.88%, Cl--34.00%; found: C--40.97%, H--4.10%, Cl--34.15%.

In a previous experiment, similar reaction conditions were used with the following differences in work-up production:

1. No additional Cl_2 was passed through the reaction mixture after it had warmed to room temperature.

2. The reaction mixture was not allowed to stand at room temperature over night to allow complete precipitation of NaCl.

3. Methanol, rather than ethanol, was used as a solvent for the second recrystallization.

Under the conditions used for the previous experiment, a product containing 5% to 6% NaCl was obtained. This appeared to be the only impurity since its infrared spectrum was identical to the pure α-chloro-2-pyridinealdoxime methchloride described above (Sample A). Elementary analysis of the slightly impure product (Sample B) was as follows:

Calculated: C--40.75%, H--3.88%, Cl--34.0%; found: C--34.6%, H--3.8%, Na--4.9%.

4.28 Preparation of Glycyl-Phenylalanine p-Nitroanilide Hydrobromide (Mel-291)

One gram of N-carbobenzoxyglycyl-phenylalanine, 0.386 g of p-nitroaniline, and 0.565 g of dicyclohexylcarbodimide were dissolved in 80 ml of dry tetrahydrofurane and were kept at 35° for 3 days. The reaction mixture was cooled in the refrigerator and filtered from the insoluble N,N' -dicyclohexyl urea. The solvent was evaporated under reduced pressure, and the residual oil was dissolved without further purification in 13 ml of 30% HBr in glacial acetic acid. After 1 hour, the solution was poured into 100 ml of dry ether.

Droplets of oil condensed at the wall of the flask and solidified. The liquid was decanted and replaced by fresh dry ether to give a nice yellow-brown solid. The solid was washed several times with dry ether, filtered, and immediately dried in a vacuum desiccator. Yield--1.15 g (96%).

Characterization:

Microanalysis: C_{17} H_{19} N_4 O_4 Br: Calculated: C--48.2%, H--4.49%; found: C--48.16%, H--6.63%.

Reference:

Analyt. Biochem. 5, 360 (1963) cf. Rev. Biochem. and Biophy. 95, 271 (1961).

Note:

An earlier batch, No. 2814-71, with 0.7 g carbobenzoxyglycylphenylalanine gave less yield. Evidently the ethanol used for recrystallization was not anhydrous enough. The solid is darker than the one described above; however, it gave the right analysis:

Calculated: C--48.2%, H--4.49%; found: C-47.7%, H--5.7%.

5.5 Preparation of 2-Amino-3-N,N Dimethyl Amino Fluorenone-9 (Mol-300)

Several small batches have been run. Only one of the examples will be given here.

2-Nitro-3-Bromo Fluorenone-9: Thirty five g of 2-amino-3-bromo fluorenone-9 were refluxed for 3 hours in 130 ml of 38% peracetic acid. The resulting solid was filtered, washed with H_2O and ethanol, and dried to give 25.0 g of 2-nitro-3-bromo fluorenone,--m.p. 250-3°--(lit. 254°).

2-Nitro-3-Dimethylamino Fluorenone-9: Ninety five hundredths gram of 2-nitro-3-bromo fluorenone-9 was dispersed in 50 ml of dimethylamine in 20 ml of ethanol, then mixed and heated in an outoclave to 70°C for 2-1/2 days. The autoclave was then shaken for 4 hours at room temperature. The precipitated crystals (with a metallic gold-brown color) were filtered and washed with H_2O and EtOH--m.p. 233-4°, 0.5 g. After evaporation of the mother liquid, 0.1 g more material was obtained. Solubility: Almost insoluble in EtOH, MeOH, AcOEt, Et_2O, hexane, dioxane, and THF; slightly soluble in AcOH, acetone, DMF and N-methylpyrrolidone:

$C_{15}H_{12}N_2O_3$: Calculated: C--67.2%, H--4.5%; found: C--66.9%, H--4.8%.

2-Amino-3-Dimethylamino-Fluorenone-9: One tenth gram of 2-nitro-3-dimethylamino-fluorenone-9 dissolved in 80 ml of glacial acetic acid was hydrogenated at room temperature (Pt-on charcoal) for 16 hours without applying pressure. The filtered solution was evaporated under reduced pressure. The residue dissolved in acetone, and a solid was precipitated by addition of H_2O. This solid was dissolved again in acetone and precipitated with hexane. The purple crystals melted at 133-5° (0.05 g):

$C_{15}H_{14}N_2O$: Calculated: C--75.6%, H--5.9%; found: C--74.5%, H--6.1%.

5.6 2-Nitro-3-Dimethylamino-Fluorenol-9 (Mel-263)

Five and three tenths grams of 2-nitro-3-dimethylamino-fluorenone-9 (described under subsection 5.5) and 35 ml of a Al-isopropoxide solution [prepared by refluxing 9 g of Al in 120 ml of isopropanol (and a trace of $HgSO_4$)] were refluxed in 54 ml of benzene for 2-1/4 hours. The hot solution was filtered to give 1.4 g starting material back. The mother liquor was acidified and extracted with 50 ml of benzene. The aqueous acidic solution was made basic and was extracted also with benzene. The combined benzene extracts were dried and evaporated to give 3.65 g of an orange solid--m.p. 148°-50°:

$C_{15}H_{14}N_2O_3$: Calculated: C--66.7%, H--5.2%; found: C--65.9%, H--5.2%.

6-Dimethylamino-7-Nitro-Phenanthridine: One and ninety five hundredths grams NaN_3 suspended in 30 ml of $CHCl_3$ was cooled to 0°. Under stirring, 7.8 ml of 98% H_2SO_4 (made from commercial H_2SO_4 and SO_3) was added dropwise. Stirring was continued for 10 minutes. The ice bath was then replaced by a bath of 25°. Under vigorous stirring, 5.2 g of 2-nitro-3-dimethylamino fluorenol-9 was added as a powder in small portions. Stirring was continued for 1-1/2 hours. The clear solvent was decanted from the sticky product. Three hundred grams of ice was added to the sticky product, and after the ice was melted, the solution was made basic, and the red solid formed was filtered (4.9 g). Nice red crystals could be isolated by recrystallization from a little hot acetone. (Four tenths gram of material was insoluble.) The addition of Et_2O to the mother liquid gave more material. The total amount of recrystallized material was 3.95 grams:

Analysis of $C_{15}H_{13}N_3O_2$: Calculated: C-67.5%, H--4.8%, N--15.7%, O-12.0%; found: C-66%, H--5.3%, N--15.5%; found (Edgewood Arsenal): C--66.6%, H--5.0%, N--15.4%, O--12.1%.